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Genetic contributions to cognitive ageing and structural brain magnetic resonance imaging phenotypes

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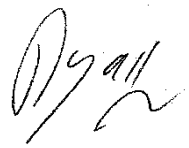
3. The work has not been submitted for any other degree or professional qualification except as specified.



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For Lilly and Bill

Thesis Abstract

As humans age, specific mental faculties deteriorate even in the absence of dementia. Age related cognitive decline affects quality of life, and has significant implications from a socio-economic perspective; however not everyone declines to equal degrees, at equal rates, or from the same baseline. This PhD examined a large sample of community-dwelling older adults called the Lothian Birth Cohort 1936, most of whom completed an intelligence test at age 11 years, and again around age 73 as part of a detailed assessment that also included detailed brain magnetic resonance imaging (N range = 700-866). I investigated the independent effects of two linked genetic loci which have been associated with greater risk of Alzheimer's disease – the *APOE* ε haplotype (commonly 'genotype') and a poly-T repeat in the *TOMM40* gene. Are 'risk' variants in these loci associated with specific measures of cognitive ageing and brain structure - specifically white matter microstructural integrity, hippocampal volumes, white matter lesions or cerebral microbleeds – in this sample?

Firstly, a pilot study aimed to replicate significant associations between the *ADRB2* gene and brain imaging/cognitive phenotypes, that had previously been reported in a smaller subsample of the cohort that had by that time undergone MRI (n = 132). Previously reported significant associations were not significant in the larger, full LBC1936 sample (n = 700-866), but novel significant associations were found ($P < 0.05$). Specifically, integrity of the left arcuate fasciculus white matter tract significantly mediated part of the association between specific genetic variations at *ADRB2*, and the Digit Symbol Coding task of information processing speed. These findings indicated that this approach – testing three-way genetic/brain imaging/cognitive associations for mediation - was viable for the main *APOE*/*TOMM40* analyses.

Results in the main *APOE*/*TOMM40* analyses showed that specific variants in the *APOE* and *TOMM40* gene loci were statistically significantly associated (at raw *P* value <

0.05) with white matter tract microstructural integrity, but not white matter lesions, hippocampal volume or cerebral microbleeds. Inconsistencies with previous, positive reports showing significant associations between *APOE* ε and these latter phenotypes may reflect a degree of type 1 error or more study-specific discrepancies (which are detailed throughout). *APOE* ε was significantly associated with average scores on a large proportion of cognitive tests, independent of age 11 intelligence (i.e. 'cognitive ageing'; Deary et al., 2004). These associations were partly – but not completely – mediated by white matter tract microstructural integrity. *TOMM40* poly-T repeat genotype was associated with cognitive ageing to a much lesser extent. A range of brain phenotypes may form the anatomical basis for significant associations between *APOE* genotype and cognitive ageing, among which includes white matter tract microstructural integrity.

Glossary

'523'	rs10524523
Aβ	Amyloid beta (plaques)
AD	Alzheimer's disease
ADRB2	Adrenergic beta-2 receptor
APOE	Apolipoprotein-e
ANOVA	Analysis of variance
BRIC	Brain Research Imaging Centre, Edinburgh.
C.I.	Confidence interval
D.F.	Degrees of freedom
DTI	Diffusion tensor imaging
FA	Fractional Anisotropy
FDR	False discovery rate

g	General factor of cognitive ability
g_{FA}	General factor of fractional anisotropy
GLM	General linear model
g_{speed}	General factor of information processing speed
g_{memory}	General factor of memory
HC	Healthy controls
ICV	Intracranial volume
LBC	Lothian birth cohort
M	Mean
MD	Mean diffusivity
MHT	Moray House Test no.12
MMSE	Mini mental state exam
MRI	Magnetic resonance imaging
NART	National adult reading test
OR	Odds ratio
PCA	Principal components analysis
RT	Reaction time
S/L/VL	Short/Long/Very-long (*TOMM40* alleles)
SD	Standard deviation
SMS1947	Scottish mental survey 1947
SNP	Single nucleotide polymorphism
TBV	Total brain volume
TOMM40	Translocase of outer mitochondrial membrane 40
η^2	Partial eta squared
WMS/WAIS	Wechsler memory scale/adult intelligence scale

Chapter 1: Introduction

1.1. Overview

On average, there is some age-related decline of specific mental functions in humans. Non-pathological mental decline – 'cognitive ageing' - has significant implications for individuals and their families in terms of quality of life, and for society as a whole in terms of financial burden (Hofer and Sliwinski, 2000; Mirkin and Weinberger, 2002; Deary et al., 2007). Individuals vary in terms of older age cognitive ability, but also vary in terms of baseline childhood ability and rate of change. It is important to understand the risk factors, modifiers and anatomical substrates of this change (Deary et al., 2007). By examining genetic contributions to cognitive ageing and structural brain magnetic resonance imaging (MRI) phenotypes in a relatively large sample of community-dwelling older adults, this thesis aims to contribute to understanding of these mechanisms.

The following sections outline the broad context of this PhD thesis (i.e. the ageing population), followed by a more specific background to the cognitive, imaging and genetic variables investigated, and the aims, dataset and outline of this thesis.

1.2. Context

1.2.1. An ageing population

The life expectancy of the world's population is increasing. In 1950, there were 205 million older adults globally, compared with 606 million in the year 2000, and a predicted two billion in 2050 (i.e. aged ≥ 60 years; UN World Population Ageing report, 2002). This is due to a number of factors including but not limited to: improvements in healthcare, home/working environments, health and safety legislation, and dissemination of health information (e.g. anti-smoking, pro-exercise advertisements; Whalley and Smyth, 2013).

The UN World Population Ageing 1950-2050 report (2002) examined data taken from several sources of official worldwide population data. This report suggests that a range of factors (as described above) contribute to two main driving forces behind population ageing:

1. Total fertility rate has decreased, e.g. between the periods 1950-1955 (2.8 children born per woman in developed regions) and 2000-2005 (1.5 children).
2. Life expectancy rates have increased, e.g. between the periods 1950-1955 (average global life expectancy = 46.5 years at death), and 2000-2005 (66.0 years).

This is indicative of a demographic shift where, as the 'baby boom generations' age, and with fertility rates low, the proportion of older adults relative to other demographics becomes significantly greater (UN World Population Ageing report, 2002). Using similar data up to 2006, Lutz et al. (2008) predicted proportionate increases in the older aged population (i.e. ≥60 years) in North America from 0.16 (out of 1; relative to the entire population) in the year 2000, to 0.18 in 2010, 0.23 in 2020, and 0.27 in 2030.

1.2.2. The implications of an ageing population

The UN World Population Ageing report (2002) describes two major consequences of a demographic shift towards an ageing population:

1. The ratio of individuals that are in some way dependent upon others will increase significantly, based on the assumption that all persons under 15 or over 65 years of age will require at least some form of support.
2. Proportionally few older adults are likely to be in a position to work due to physical health limitations.

This is likely to impact significantly upon economic growth due to reduced labour and output coupled with increased healthcare and general support costs (e.g. out-patient dependency programs, disabled access; Zhao et al., 2013).

1.2.3. Cognitive ageing

Successful ageing extends beyond the absence of mental or physical illness to include personal independence and autonomy; this requires relatively intact mental faculties (World Health Organisation, 2003; Fiocco and Yaffe, 2010). Specific cognitive abilities such as attention and memory are relevant for higher-order skills such as planning and decision making, and these are important in terms of maintaining independence and a good quality of life – for example, remembering to take medication, prepare meals safely, and manage finances (Fiocco and Yaffe, 2010; Burton et al., 2007). Losing mental function is among the most feared aspects of ageing (Martin, 2004).

What factors influence the natural age-related decline in a person's ability to process, manipulate, recall and communicate complex information? Individuals vary along a continuum of cognitive ageing, and past studies have examined a range of potential risk factors including but not limited to: cardiovascular disease pathologies, specific genetic variables, levels of education, and smoking/alcohol/dietary intakes, among other potentially important variables (Korczyn and Vakhapova, 2007; Deary et al., 2009; Plassman et al., 2010).

Cognitive ability in older age plays a significant role in maintaining independence and quality of life. This is important from both compassionate and socioeconomic perspectives, and it is therefore critical to understand the risk factors and anatomical brain substrates underlying cognitive ageing (Treves and Korczyn, 2011; Deary et al., 2007).

1.3. Thesis background

1.3.1. Specific cognitive abilities are more sensitive to age

Cognitive ability can be fractionated into different domains, such as memory or reasoning. This observation is based on statistical methods such as factor analysis of different cognitive tests (e.g. Carroll, 1993; Salthouse, 2010; Penke et al., 2012), and on patterns of neuropsychological impairment in groups of participants with specific brain lesions (i.e. preservation of some cognitive abilities, but not others; e.g. Della Sala et al., 2004).

There is evidence that specific aspects of cognitive ability are more sensitive than others to the effects of age. For example, tasks of 'fluid' intelligence, e.g. executive functions, information processing speed, declarative memory, are more affected by age compared with aspects of 'crystallized' intelligence (i.e. general semantic information such as vocabulary), which are generally relatively resilient to age (Horn & Cattell, 1966; Salthouse, 2010; 2011). In evidence of this, Wilson et al. (2002) examined 694 older adults in the Religious Order Study, a longitudinal assessment of older Catholic nuns, priests and brothers. The participants showed no evidence of Alzheimer's disease (AD), in that they all scored above 24 on the Mini Mental State Exam, a common screening tool for dementia (out of 30; MMSE; Folstein et al., 1975). Participants were of varying ages (mean age = 75.9 years, standard deviation [SD] = 6.9), and they completed a range of tests including declarative memory (split into 'Story retention' and 'Word retention'), 'Word knowledge' (reflecting crystallized intelligence), working memory, 'Perceptual speed' (i.e. information processing) and visuospatial tasks (21 tests in total). They found greater effects of cross-sectional age at time of assessment for fluid-type tests (Story retention r correlation with age = -0.29, Word retention r = -0.37, Working memory r = -0.30, Perceptual speed r = -0.42, Visuospatial ability r = -0.34), than for Word knowledge (r = -0.10; reflecting crystallized ability; specific P value not provided).

1.3.2. Cognitive ageing is modified by different variables including specific genetic loci

Cognitive ageing may be modified by a number of variables, including environmental or genetic risk factors (Haan et al., 1999). Evidence of a significant role for genetic variation on cognitive ageing is provided in a large study by Davies et al. (2012). Davies et al. conducted a genome-wide association study on cognitive ageing based on five large discovery cohorts (Lothian Birth Cohorts 1921 & 1936 [LBC1921/1936]; Aberdeen Birth Cohort 1936 [ABC1936]; Manchester and Newcastle Longitudinal Studies of Cognitive Ageing Cohorts [the University of Manchester Age and Cognitive Performance Research Centre; ACPRC, and North East Ageing Research; NEAR cohorts, respectively]), totalling 3802 participants. Significant associations were re-tested in two replication cohorts (total N = 1367). General factors of cognitive ability were created for each of the cohorts, using different tests of fluid intelligence, by principal components analysis (PCA). Each score was then adjusted for premorbid ability; either with measures of childhood intelligence (LBC1936/1921 and ABC1936), or with growth curve models based on data from up to four time points, including ability at 10 years prior (ACPRC/NEAR cohorts). Older age ability adjusted for prior ability was considered 'cognitive ageing'. Participants in the discovery groups were genotyped for 549,692 common single nucleotide polymorphisms (SNPs) plus a bespoke assay for apolipoprotein (*APOE*) ε2/ε3/ε4 status. They found that possession of the *APOE* ε4 allele (vs. possessing only ε2 or ε3 alleles) was associated with significantly worse cognitive ageing (unstandardised β = -0.22, standard error = 0.04, $P = 2.18 \times 10^{-8}$), and that no other SNP showed a significant association once variation at *APOE* ε was controlled for (Davies et al., 2012).

1.3.3. The anatomical brain substrates of cognitive ageing

There is evidence that fluid-type intelligence tests tend to be more sensitive to the effects of age, compared with more crystallized-type tasks (Wilson et al., 2002; Salthouse, 2011). A logical extension of this is that specific brain substrates are more sensitive to age, and underpin these significant fluid-type cognitive associations with age. Schretlen et al. (2000) examined how frontal lobe volumes underpinned associations with cognitive ability. In 112 participants (mean age = 54.0, SD = 19.0) detailed cognitive and brain MRI assessments were performed. They constructed crystallized and fluid-intelligence 'factors' using principal components analysis (PCA). Based on eight tests of semantic (e.g. Information) and fluid-spatial (e.g. Block Design), ability, they generated two factors: a crystallized ability factor that accounted for 36.7% of the variance (*r* with age = 0.64*, r* with education = 0.66), and a fluid ability factor that accounted for 31.1% of the variance (r with age = -0.56, *r* with education = 0.32). They found that frontal lobe volumes correlated significantly with age (*r* = -0.28, *P* = 0.003), but overall brain volume minus frontal volume did not (*r* = 0.16, *P* = 0.100). Comparing models for the fluid factor, Schretlen et al. found that a model with age (β = -0.39, *P* < 0.001) was improved significantly by adding frontal lobe volume (β = -0.27, *P* <0.001, total variance explained = 61%, an increase of 37%). Therefore the model was significantly improved by adding frontal lobe volumes (F [2, 109] = 32.6, *P* <0.001). This was not the case with brain volume minus frontal lobe volume (all *P* > 0.05), indicating a degree of frontal lobe structure-function specificity.

There are limitations to the above (and similar) studies which investigate anatomical substrates to specific cognitive abilities. Each specific brain imaging phenotype is only partially informative and a range of brain imaging phenotypes are therefore required to elucidate the more precise underpinnings of the specific cognitive abilities tested. This also means that only relatively large sample sizes are likely to provide reliable results (Salthouse,

2011). Penke et al. (2010a) for example investigated the role of white matter integrity using different metrics, one of which was fractional anisotropy (FA). White matter is characterised by a fatty myelin layer that permits efficient communication between brain regions, and is therefore a plausible substrate of cognitive functioning (Penke et al., 2010a). Penke et al. reported that a general factor of white matter tract integrity FA (g_{FA}), constructed with principal components analysis (PCA), was significantly associated with a general factor of information processing speed (r = -0.24, P = 0.007) in a subsample of the LBC1936 dataset that had by that time undergone diffusion tensor MR imaging (DTI; n = 132; aged around 73 years). In a later study examining a larger sample of the LBC1936 cohort, g_{FA} significantly associated with general fluid-type cognitive ability (g; standardized β = 0.13, $P < 0.05$) explaining around 10% of the variance (n = 420; Penke et al., 2012).

1.4. Genetic variables

To recap, cognitive ageing is affected by a number of variables including genetic factors, one of which may be the *APOE* locus, and it is possible that these associations occur via intermediate brain imaging phenotypes. It is possible that other variables – possibly genetic - moderate the effects of *APOE* or exert independent effects. This section details *APOE* in addition to a linked, possibly modifying locus in the translocase of outer mitochondrial membrane *40 (TOMM40)* gene, and their collective relevance to brain ageing.

1.4.1. Apolipoprotein-e (APOE)

The *APOE* gene is located on chromosome 19q13.2 and is 3.7 kilobases (KB) long. The *APOE* ε haplotype (commonly and herein referred to as 'genotype') is composed of two SNPs; rs429358, which causes a Cys130Arg substitution; and rs7412, which causes an

Arg176Cys substitution (http://www.ncbi.nlm.nih.gov/gene/348). Different combinations of the rs329358/rs7412 SNPs form the ε2 (Cys/Cys respectively), ε3 (Cys/Arg) and ε4 (Arg/Arg) genotypes (Ringman and Cummings, 2009). Of these, the ε3 allele is the most common (frequency ~78.3%), followed by ε4 (~14.5%) and ε2 (~6.4%), although this can vary by population (Eisenberg et al., 2010).

APOE plays a role in the transport and metabolism of lipid in the human body and brain (Corder et al., 1994; Bu, 2009). The lipid family includes cholesterols, sphingolipids, fatty acyls, glycerolipids, glycerophospholipids, cardiolipids, sterols, triglycerides and high/low density lipoproteins, and is important for energy storage and maintaining the structure and function of cellular membrane (Fahy et al., 2008). The ε4 allele preferentially binds to large lipoprotein particles (Bu, 2009), and for several phenotypes is the 'risk' variant compared with ε3 ('neutral'), and ε2 ('protective', although less consistently). These phenotypes include: risk of Alzheimer's disease (AD) (Corder et al., 1994); less successful cognitive ageing (Deary et al., 2004; Wisdom et al., 2011); differences in brain structure e.g. atrophy (Biffi et al., 2010) and functional connectivity (Trachtenberg et al., 2012); and cardiovascular pathologies such as hyperlipidaemia, coronary heart disease and stroke (Stenset et al., 2006). It is not clear to what extent associations between *APOE* ε4 and worse cognitive ageing reflect preclinical 'prodromal' AD or mild cognitive impairment (MCI) (Bretsky et al., 2003; Deary et al, 2004).

The majority of associations between single common candidate gene variants (i.e. with minor allele frequencies above 5%) and complex disease traits do not replicate in independent samples due to inadequate power, differences in study design/analytic strategy and low prior probability of true association; any one candidate gene locus is only likely to explain a small amount of total phenotypic variation (~4-5%; Hattersley and McCarthy, 2005; Munafo et al., 2006). *APOE* ε4 has been significantly associated with greater risk of

AD in a number of independent samples, and is a generally accepted risk factor for AD (Corder et al., 1994; Hardy and Higgins, 1992; Hardy, 2006). For example Farrer et al. (1997) demonstrated significant association between *APOE* ε4 and AD in several large samples. They examined 40 independent samples, including individuals diagnosed with AD, and healthy controls, stratified by race into Caucasian (sample N's = 5107 vs. 6262, respectively), African American (N = 235 vs. 240), Hispanic (N = 261 vs. 267), and Japanese (N = 336 vs. 1977) analyses. Statistically adjusted for age, gender and cohort membership (i.e. research centre), in the Caucasian sample they found significant deleterious effects of the ε2/ε4 (odds ratio [OR] = 2.6, 95% C.I's = 1.6 to 4.0), ε3/ε4 (OR = 3.2, 95% C.I's = 2.8 to 3.8), and ε4/ε4 (OR = 14.9, 95% C.I's = 10.8 to 20.6), vs. the neutral ε3/ε3 genotype. These results were similar in different ethnicities, reflecting deleterious effects of the ε4 allele.

The *APOE* ε4 allele may contribute to phenotypic differences through different and possibly interactive mechanisms:

1. By promoting the build-up and inhibiting the clearance of amyloid-beta plaques in the brain. Accumulations of amyloid-beta disrupt synaptic transmission, and along with high levels of neurofibrillary tangles (NFT) this pathology is most characteristic of clinical AD (Martins et al., 2006; 'Amyloid Cascade Hypothesis', Hardy and Higgins, 1992).
2. Through secondary association with cardiovascular pathology. This could be by hindering communication between the heart and brain (specifically known as cerebrovascular disease e.g. atherosclerosis; Erkinjuntti, 2007; De la Torre, 2010). Cardiovascular diseases such as hypertension are common risk factors for cerebrovascular disease (De la Torre, 2010)

3. Disrupting the effective transport of cholesterol. This is essential for the development and maintenance of neurons and the myelin sheaths that characterise white matter (Ringman and Cummings, 2009; Martins et al., 2006; Hooijmans and Kiliaan, 2008).

It is thus generally agreed that *APOE* ε4 genotype is a significant risk factor for AD pathology, yet the association with clinical AD or cognitive decline is far from perfect (Johnson et al., 2011). There is heterogeneity in cognitive decline that *APOE* genotype does not account for, and which may be explained by other factors (Crenshaw et al., 2013). Other genetic variants aside from *APOE* may play a role.

1.4.2. Translocase of Outer Mitochondrial Membrane 40 (TOMM40)

Mitochondrial dysfunction may play a significant role in cognitive decline and AD pathology ("mitochondrial cascade hypothesis"; Swerdlow et al., 2010). The mitochondrial cascade hypothesis is based on the observation that the amyloid cascade hypothesis fails to account for physiological changes in people diagnosed with AD, that are not limited to the brain – such as reduced cytochrome oxidase activity, greater free radical production and evidence of greater oxidative stress (Kish et al., 1992; Parker et al., 1990). The amyloid cascade hypothesis also fails to consider that amyloid precursor protein and amyloid-beta accumulations that are similar to those found in clinical AD can also be found in people with traumatic brain injury, suggesting they are actually a secondary reaction or response to separate, more primary deleterious changes in the brain (Reitz, 2009).

The mitochondrial cascade hypothesis is based on certain core assumptions (adapted from Swerdlow et al., 2010):

1 Baseline mitochondrial function, viability and rate of natural decline are determined by nuclear genetic and environmental variables.

2 Decline in mitochondrial function drives age-related change in a number of biological phenotypes, but is compensated for throughout the lifespan through changes in cell physiology.

3 Histological changes typical of AD such as amyloid-beta pathology begin to manifest during the compensatory phase.

4 This compensation fails at a certain point, and histological changes that more closely resemble clinical AD begin to manifest as a consequence, including tau phosphorylation, synaptic loss, cell cycle re-entry and latent neurodegeneration.

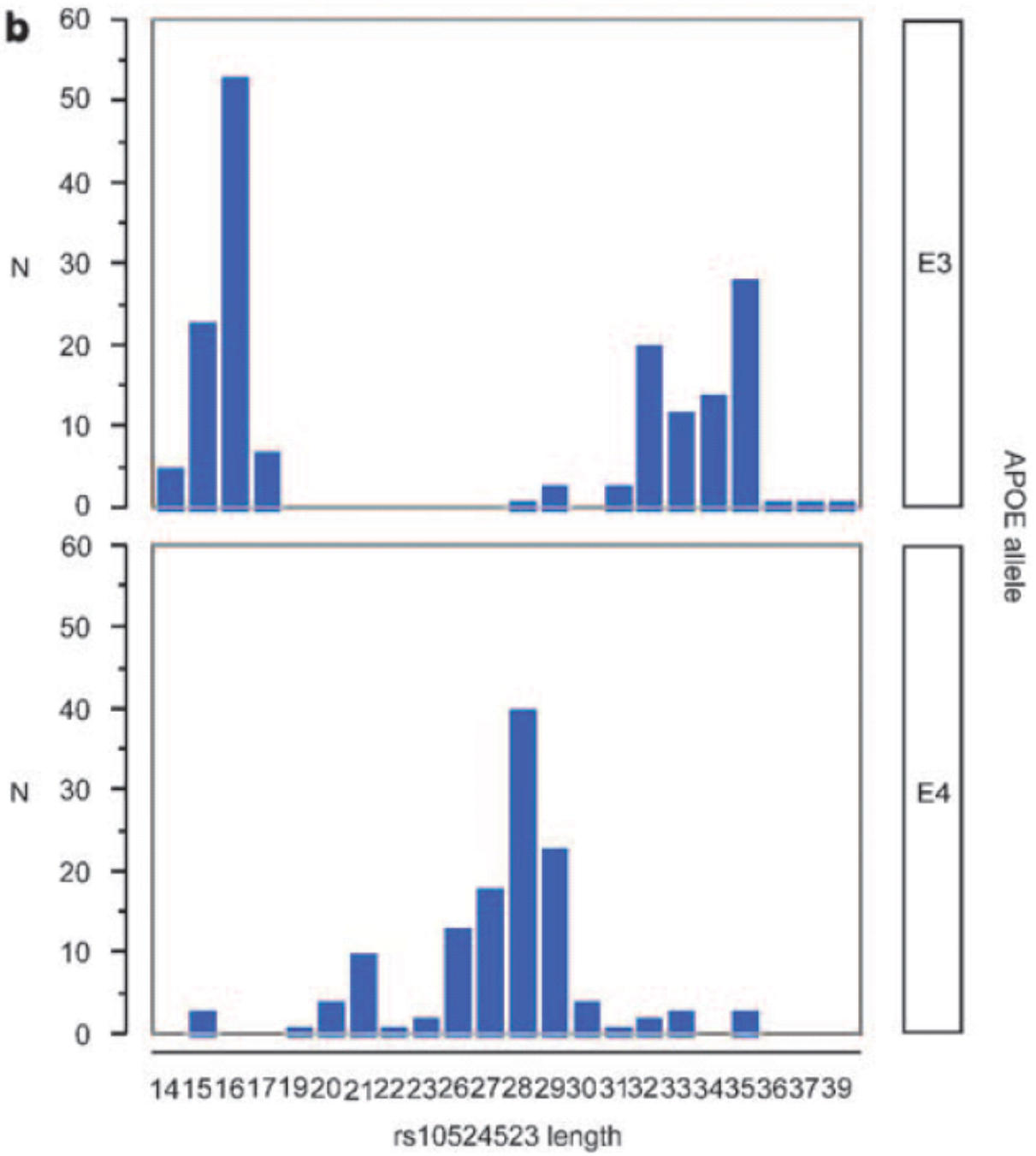
5 The above assumptions explicitly consider common factors to be underlying age-related changes and also AD. In terms of the pathological processes leading to AD, the mitochondrial cascade hypothesis considers mitochondrial dysfunction to occur earlier than the amyloid-beta accumulations found in clinical AD (Ferencz et al., 2012).

TOMM40 encodes the channel-forming subunit of the translocase of outer mitochondrial membrane (TOM) complex (http://www.ncbi.nlm.nih.gov/gene/10452; Humphries et al., 2005). This complex imports precursor proteins into mitochondria (Koehler et al., 1999). Specifically, precursor proteins in the cytosol first bind to import receptors Tom-20 and Tom-70 on the mitochondrial surface. The channel-forming Tom-40 is then the basis of a 'core complex' (together with the smaller receptors Tom-22, -7, -6 and -5), which creates a protein-conducting pore that transports precursors across the outer membrane, into mitochondria (Rapaport, 2005). It has been suggested that *APOE* and *TOMM40* may interact to affect mitochondrial dynamics although mechanistically it is unclear exactly how this may occur (Roses et al., 2010).

The rs10524523 locus (hereafter '*523*') in *TOMM40* is characterised by a variable number of T residues (poly-T repeats) that can be grouped into 'Short' (<20; 'S'), 'Long' (20-29; 'L'), and 'Very-Long' (≥30; 'VL') (Lutz et al., 2010). Roses et al. (2010) plotted

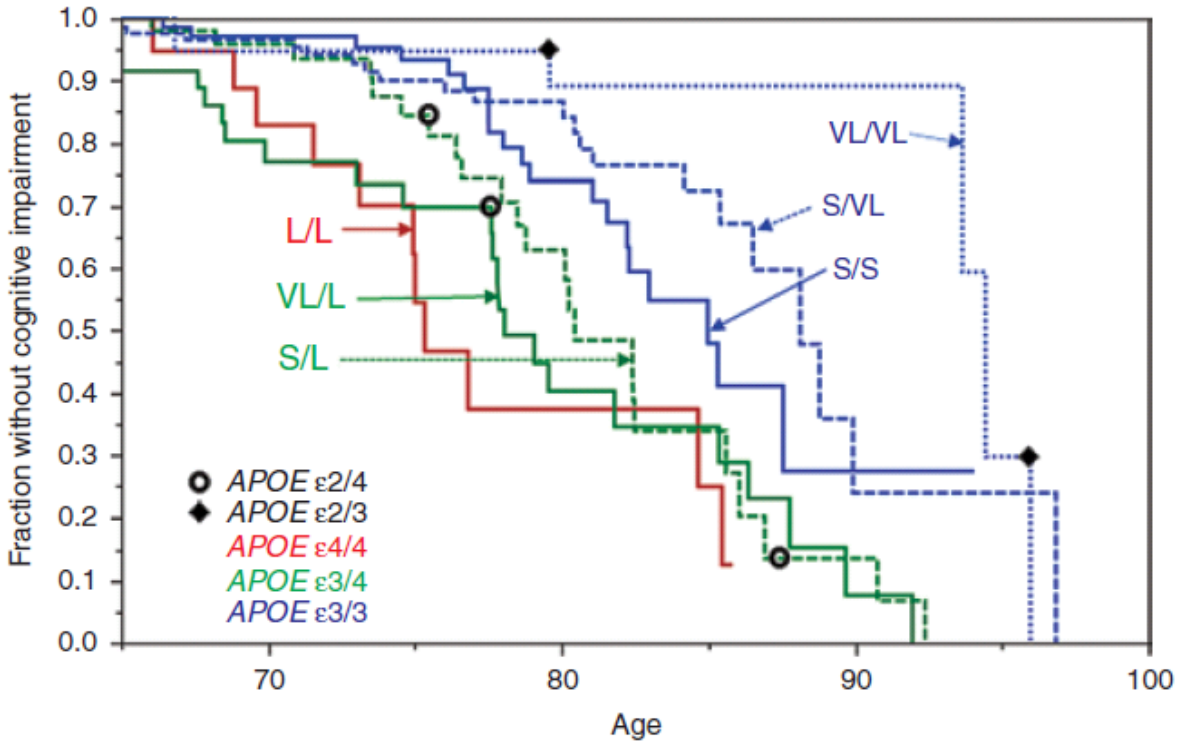
histograms showing the distributions of poly-T repeat lengths in different *APOE* genotypes; ε3/ε3, ε3/ε4 and ε4/ε4. The poly-T repeat was strongly linked with the *APOE* ε haplotype; ε4 is linked to L, with ε3 linked to either S or VL alleles (Roses et al., 2010; Figure 1.1). The rarer ε2 allele appeared to show similar linkage to S or VL alleles in *TOMM40* 523 - as per ε3 - although further research is required (Roses et al., 2010).

Figure 1.1. Frequencies of TOMM40 '523' poly-T repeat lengths stratified by APOE ε3 (top) and ε4 alleles (taken from Roses et al., 2010).



The *TOMM40* 523 repeat is of particular interest because a large number of SNPs in the *APOE/TOMM40* region are in significant linkage disequilibrium (e.g. rs429358 and 36 SNPs within ±1.17 KB of the *APOE* region including 15 *TOMM40* SNPs; average D' = 0.91, r^2 = 0.22, N = 1262; Takei et al., 2011). This means that it is difficult to extrapolate effects of SNPs in the region that are truly independent from *APOE* ε4 (Hong et al., 2010; Davies et al., 2012). In contrast, recent studies have reported effects of *TOMM40* 523 independent of *APOE* ε genotype. An illustrative example comes from Crenshaw et al. (2013). They examined 508 generally healthy, non-demented older adults (mean age = 74.0, SD = 9.5; baseline MMSE score mean = 29.0, SD = 1.3) that were cognitively assessed prospectively on fluid-type cognitive tasks considered sensitive to the effects of age (Weintraub et al., 2009; Morris et al., 2006). They examined how *TOMM40* 523 genotype influenced the prospective onset of cognitive impairment (i.e. marked decline in performance based on a range of cognitive abilities; conversion events = 106). They stratified the sample by *APOE* ε; keeping this constant allowed Crenshaw et al. to test for biologically independent effects of *TOMM40* 523. Crenshaw et al. observed marked variation in the onset of impairment with age, according to *TOMM40* 523 genotype. Specifically, note that in *APOE* ε3/ε3 genotype subgroup, the S/S group has proportionally greater and earlier onset of cognitive impairment compared with the VL/VL genotype group, with S/VL intermediate. Although Crenshaw et al. did not test for significance, Figure 1.2 demonstrates the heterogeneity that may exist according to *TOMM40* 523 genotype, when *APOE* ε genotypes are kept stable.

Figure 1.2. Fractions of older individuals (baseline N = 508) that are classified as having cognitive impairment, stratified by APOE genotype groups, to illustrate the independent effect of TOMM40 '523' poly-T repeat length (S = Short; L = Long; VL = Very-long). Taken from Crenshaw et al. (2013)



Recent studies have shown relatively consistent, significant associations between *TOMM40 523* genotype and brain-related phenotypes such as age of AD onset (Roses et al., 2010), and brain phenotypes such as medial temporal lobe gray matter volumes (Johnson et al., 2011), independent of *APOE*. These reports are detailed in Table 1.1. Reports vary in showing protective, null or deleterious effects of the S allele (e.g. Johnson et al., 2011; Chu et al., 2011 & Cruchaga et al., 2011 respectively). These conflicting reports suggest that investigations of the *TOMM40 523* poly-T repeat should not make a-priori predictions regarding direction of association.

The exact functional significance of *TOMM40* 523 poly-T repeat length is a matter of ongoing research. Bekris et al. (2011; N = 32) conducted functional analysis of different poly-T repeat haplotypes on reporter assay expression levels in SHSY5Y, HepG2 and U118 neuronal cell lines. Results indicated that specific *TOMM40* 523 haplotypes were associated with lower *TOMM40* – but not *APOE* – gene expression in SHSY5Y neuronal cell lines only, indicating a role for this locus in *TOMM40* promoter silencer/enhancer activity, but that the direction of its effect on expression depends on the cell type and specific haplotype content. Hedskog et al. (2012) assessed the biochemical properties of different poly T repeat lengths in fibroblast cell cultures taken from *APOE* ε3/ε4 carriers (S/L vs. VL/L only, n = 8). They observed no significant differences between groups in terms of: i) *TOMM40* gene mRNA splicing, stability or expression, ii) protein expression levels of *APOE*, *TOMM40* or Presenilin-2 (*PSEN2*; linked to familial AD), or iii) transcription/translation of the *TOMM40* protein. Additionally, they found no evidence of poly-T repeat lengths affecting mitochondrial function (assessed by 'ΔΨm', reflecting depolarization of transmembrane potential), morphology or biogenesis (indicated by mitochondrial volume density per fibroblast cell). Similarly, in a sample of healthy (n = 39) and AD participants (n = 82), Cruchaga et al. reported that poly-T repeat length was not significantly associated with protein expression as measured using complementary DNA obtained from the parietal lobe. (Further study of poly-T repeat function and significance – beyond these detailed studies - is required in large samples, including those with AD, *APOE* data, and age of onset data.)

Table 1.1. Previous reports investigating the translocase of outer membrane 40 *(TOMM40)* '523' gene locus and cognitive/neuroimaging phenotypes.

Authors	*Phenotype (TOMM40 analysis)*	*Sample (mean age ±SD)*	*N*	*Covariates*	*Genotype-phenotype associations*	*Type 1 error adjustment*	*Notes & limitations*
				Protective effects of Short allele			
Bruno et al. 2011a	Neurofilament light protein levels in CSF (ng/l); a marker of neuronal damage where ↓ = possible axonal loss. (S+ vs. S-, stratified by *APOE* ε4+ & ε4-).	Healthy older adults (67.1 years ±6.2 years*).	47	MMSE score, ender, major depressive disorder, education (statistically controlled).	In ε4+: a deleterious effect of S- *(S+ M [Mean] = ~550, range = 270-1400, S- M = ~900, range = 240-1740, P = 0.033).* In S-: protective effect of ε4 ↑ *(ε4+ M = ~900, range = 240-174, & ε4- M = ~450, range = 180-820, P = 0.018) (data estimated from figures.*	Fishers LSD	Very small sample; S+ n = 30, S- n = 17. Protective effect of ε4 in non-S carriers is unexpected.
Bruno et al., 2011b	Cortisol levels in CSF (ug/dL) where ↑ = can contribute to hippocampal damage. (S+ vs. S-, in ε4+ & ε4-)	Healthy older adults (67.1 ±6.4*).	57	Age, Education, MMSE, gender, major depressive disorder (controlled).	In S-; deleterious effect of ε4 *(ε4 + M = ~0.6, ε4- M = ~0.45, P = 0.028)*, but no effect of ε4+ overall, or in S+ group.	Fishers LSD	*TOMM40* 523 poly-T repeat may modulate the toxic effects of *APOE* ε4 allele (Bruno et al.).
Roses et al. 2010	Age of AD onset in years (In ε3/ε4 carriers only; VL+ vs. VL-).	Older adults with AD (age of onset 71.2*; SD unclear).	34	None.	VL+; earlier onset of AD *(VL+ M years = 70.5 ±1.2, VL- M = 77.6 ±2.1, P = 0.02).*	Bonferroni	Very small sample. VL genotype was ≥27 poly-T repeats unlike more recent studies, which use ≥30.
Johnson et al. 2011	Gray matter volume 1. whole-brain comparison 2. age-by-523 VL allele dose interaction on volumes (In ε3/ε3 carriers only, VL dose contrast: 0 vs. 1 vs. 2).	Healthy older adults (55.3 ±6.0*; APOE ε3/ε3 genotype only).	117	Age, intracranial volume, gender (controlled), Family history of AD, hypertension (sig. higher in S/VL group).	1. volume ↓ in left ventral posterior cingulate *(t = 4.10, voxel cluster size = 796, P = 0.001)* and cuneus *(T = 3.43, cluster = 376, P = 0.042)* 2. age-by-523 interaction for volumes in retrosplenial area *(t = 3.21, cluster = 35, P = 0.001)* and right anterior mesial temporal lobe *(T = 2.83, cluster = 23, P = 0.003).*	None	Sig. increased hypertension and family history of AD in S/VL group. Deleterious effects of VL allele.
Caselli et al. 2012	Verbal memory scores. (Auditory verbal learning test).	Healthy older adults longitudinally assessed every 1-2 years (58.2 ± 12.3*).	639	Age, gender, education (n.s.; $P > 0.05$ between groups).	Significant difference in memory change between S/S and VL/VL (deleterious) groups in *APOE* ε3/ε3 group only ($P = 0.04$).	None	

Null findings

Chu et al. 2011	Age of AD onset; years (number of L alleles).	Older adults with probable/diagnosed AD (age of onset = 73.0 ±6.3)	892	None.	Association between L allele dose and age of onset *(standardised β = -0.077, P = 0.002)* attenuated to n.s. when ε4 dose controlled.	None	The largest sample investigating 523 poly-T repeat. Poly-T repeats; VL according to ≥27.
Schiepers et al. 2012	Cognitive tasks - Verbal Fluency, Logical Memory and Ravens Progressive Matrices. Age 79 scores where ↑ = better, and decline across ages 79-87 where ↑ = worse. (1. 523 genotype; main effects) (2. L+ vs. L-).	Healthy older adults; (79.1 ±0.6*). Re-tested at around 83 & 87 years.	187	MMSE (n.s), age 11 IQ, BMI, smoker status, alcohol consumption, cholesterol, cardiovascular disease history (controlled).	1. L/VL carriers (vs. other genotypes); ↓ Logical Memory (baseline) *(parameter estimate = -5.06, 95% C.I's -9.39 to 0.74, P = 0.013),* ↑ Logical Memory decline *(estimate = -0.69, 95% C.I's -1.37 to -0.01),* ↑ Ravens Progressive Matrices decline *(estimate = -0.39, 95% C.I's -0.77 to -0.01).* 2. L+; ↓ Logical Memory decline *(M = -4.18 vs. 1.06, P = 0.006).* All n.s. when controlled for ε4 alleles.	None	Greater decline in Logical Memory for L/VL vs. S/S, but smaller decline when stratified into L+ (vs. L-). This is slightly contradictory.
Pomara et al. 2011	Amyloid β1-40/1-42 & brain tau concentrations in CSF, where ↑ can interfere with synaptic function (S+ vs. S-, stratified by ε4+ & ε4-).	Healthy older adults (67.1 ±6.2).	47	Age, education, gender, MMSE, Hamilton depression scale scores (controlled).	No effects of 523 status; either by S+ vs. S-, or S/L/VL genotype. Main effects of *APOE* only.	None	Twenty-eight participants had clinical depression.

Deleterious effects of Short allele

Cruchaga et al. 2011	1. age of onset 2. risk of AD diagnosis Tested overall sample first, and then ε3/ε3 carriers only (overall 523 genotype & then *APOE*-ε/523 phased haplotype).	Healthy older adults (75.6 ±8.7*), and also older adults with AD (73.5 ±20.7* at onset).	(HC) 1190 & (AD) 1594	Age, gender (controlled), ethnicity (n.s.). Clinical Dementia Ratings healthy group score = 0, AD score > 1.	i. VL allele; later age of onset in whole sample *($P = 1.03 \times 10^{-19}$, but n.s when APOE-ε4 controlled, and no effect in ε3/ε3 or ε3/ε4 carriers*). Phased haplotype; ε3/clade A (reflecting VL allele) had later age of onset AD *(ε3-clade A M = 73.31 vs. ε3-clade B M = 72.93, P = 0.057)* ii. L allele in whole sample; trend ↑ risk of AD *(case freq = 0.41, control freq = 0.16, P = 0.08 controlling for APOE)*. VL allele in ε3/ε3; ↓ risk of AD *(case freq = 0.41, control freq = 0.48, P = 0.004;VL & AD risk OR = 0.89).*	Bonferroni	Reported significant protective effect of VL allele in terms of AD risk. The largest investigation of 523 repeat & AD phenotypes. Effects all in the opposite direction to those reported by Roses et al. 2010.
Hayden et al. 2012	Broad cognitive test battery (17 tests).	Healthy older adults (80.6± 6.0).	127	Age, gender, *APOE*, education, depression (controlled).	No significant effects in whole sample. Several significant deleterious effects of S/S vs. S/VL alleles presence for several tasks (in *APOE* ε3/ε3 subgroup only, n = 82; detailed in Chapter 7; all $P < 0.05$).	Bonferroni	Deleterious effect of S allele in 'neutral' *APOE* genotype group only; hence, small sample. Also, S/VL performed much better than S/S and VL/VL, who performed similarly. This is surprising. No associations survived bonferroni correction. No markers of prior ability.
Cruchaga et al. 2011 (above report)	AD-related phenotypes; CSF amyloid β-42 & tau (overall 523 genotype).	CSF; healthy adults (85.6 ±7.1).	733	See above.	Whole sample & ε3/ε3 carriers; no effect of 523 on Aβ42 or tau levels independent of *APOE*.	See above	Indicates that the mechanisms underlying any 523/AD association are not *APOE* routes such as Aβ42 levels.

Note APOE = apolipoprotein-E gene, *TOMM40* = translocase of outer mitochondrial membrane 40 gene, S = Short allele, L = Long Allele, VL = Very-Long allele, +/- = carrier/non-carrier, AD = Alzheimer's disease, CSF = cerebrospinal fluid, n.s. = not statistically significantly different between groups at $P > 0.05$, N/A = not available/applicable, SD = standard deviation, M = mean, C.I. = confidence interval, OR = odds ratio, MMSE = mini mental state exam, BMI = body mass index. Tau = neurofibrillary tangles. All significant findings ($P < 0.05$) survived correction for multiple testing unless otherwise stated. All ages are in years. * = weighted estimates.

1.5. Approach and aims of this thesis

To recap, the *APOE* ε gene locus is a risk factor for AD, and the ε4 allele has been significantly associated with worse cognitive ageing in large samples of older adults; it is possible that these associations occur via intermediate brain imaging phenotypes. However other genetic variants - such as the *TOMM40* 523 poly-T repeat - may modify this association and exert influence on brain phenotypes, independent of *APOE* ε (e.g. Crenshaw et al., 2013; described above).

Few studies have attempted to elucidate the anatomical brain substrates of *APOE* ε-cognitive ageing associations. This is because there are few datasets with detailed genetic, brain MRI and cognitive data, however these are available in the Lothian Birth Cohort 1936 (LBC1936; detailed below), a relatively large sample of community-dwelling older adults. Additionally, few studies have investigated the effects of *TOMM40* 523 genotype on brain imaging or cognitive phenotypes, independent of *APOE* ε.

This PhD thesis intends to expand upon the current literature through three specific aims:

1. Contribute a relatively large amount of data on the association between *APOE* ε and brain imaging/cognitive ageing phenotypes, in generally healthy community-dwelling older adults.
2. Elucidate the effects of *TOMM40* 523 genotype in brain imaging/cognitive ageing phenotypes, and tests whether they are independent of *APOE* ε.
3. Elucidate the anatomical substrates of significant associations between *APOE* ε and cognitive ageing phenotypes, with detailed structural brain MRI (statistically tested for mediation).

Before each analysis, each chapter will provide an overview and critique of relevant previous research.

1.6. Dataset used in present thesis: the Lothian Birth Cohort 1936

For the purposes of this thesis, data was examined from the LBC1936, a large sample of generally healthy, community dwelling older adults with detailed genetic, brain MRI, cognitive and sociodemographic/medical information available (Deary et al., 2007; 2012).

Most of the participants completed the Moray House Test (MHT no. 12; an intelligence-type test) around the age of 11 years as part of the Scottish Mental Survey 1947 (Deary et al., 2007). Around the age of 70 years, 1091 participants repeated the MHT in addition to a range of assessments related to cognitive and other phenotypes (as described above, excluding brain MRI; 'Wave one'). Later, around the age of 73, participants repeated this assessment and also received detailed brain MRI (Deary et al., 2007; 2012; Wardlaw et al., 2011; 'Wave two'). The data collected around the age of 73 – Wave two – are those used in the present thesis, in addition to the described measure of childhood intelligence.

An unusual and valuable aspect of the LBC1936 sample is that most participants have a measure of childhood intelligence (i.e. MHT scores) from age 11. Scores on cognitive tests completed around age 73, statistically adjusted for age 11 ability - before the effects of age-related variables such illness – therefore to an extent reflect cognitive ageing (Deary et al., 2004). The recruitment and assessment protocol for this sample is described in Chapter 2 ('*Methodology'*).

1.7. Thesis outline

The present thesis includes four empirical reports detailing relevant previous research (Chapters 4 to 7), preceded by an empirical proof-of-concept study (Chapter 3) and a methodology chapter (Chapter 2). Chapters 4-6 examine the genetic contributions of the *APOE* ε and *TOMM40* 523 loci to specific brain imaging phenotypes. Chapter 7 examines the effects of these loci on cognitive ageing, and then formally tests whether significant gene-cognitive associations are mediated by associations with brain imaging phenotypes (as reported in Chapters 4-6) which are also associated with the cognitive variables (i.e. in situations where the three-way associations all have raw $P < 0.05$). This 'Causal steps' model investigates mediation where all variables are significantly associated as this is where mediation could be expected the most (Hayes, 2009; Salthouse, 2011). Chapter 3 provides an empirical proof-of-concept for this. Chapter 2 provides the methodology common to Chapters 4-7, and also partly to Chapter 3 (which examines a different genetic locus). Chapter 8 provides a discussion and overview of the findings.

Chapter two introduces the sample used in subsequent chapters - the Lothian Birth Cohort 1936, and describes methodology common to subsequent chapters. This chapter details the genotyping and analytic strategy for *APOE* ε and *TOMM40* 523 (relevant to Chapters 4-7 but not Chapter 3), and phenotyping in terms of brain MRI, cognitive, medical and sociodemographic information. Because subsequent chapters examine different imaging/cognitive phenotypes, a number of methodological details are study-specific, and therefore reported in each respective chapter.

Chapter three examines the role of two SNPs in the adrenergic beta-2 receptor gene (*ADRB2*). Penke et al. (2010b) reported - in a smaller subsample of the dataset used in this thesis - that two SNPs were significantly associated with performance on specific cognitive tests, and these significant associations were mediated by white matter microstructural

integrity, as assessed with diffusion-tensor MRI. This chapter aims to replicate these mediating relationships in the larger, full sample of LBC1936 participants with MRI data, and expand analysis to a broader range of cognitive and white matter microstructural phenotypes. Chapter 3 is a proof-of-concept study in that it demonstrates the effects of specific, common genetic variants on cognitive ageing, mediated by brain MRI metrics; suggesting feasibility for subsequent chapters.

Chapter four examines associations between variation in *APOE* ε/*TOMM40* 523 and white matter microstructural integrity (again assessed with DT-MRI). This sensitive phenotype has been significantly associated with changes in AD (Sexton et al., 2011); the vast majority of previous reports of *APOE* are relatively small (Gold et al., 2012), and no study has investigated the effects of the *TOMM40* 523 locus on this phenotype.

Chapter five examines *APOE* ε/*TOMM40* 523 variation and hippocampal volume. This phenotype is of interest because the hippocampus is the earliest site of neurofibrillary AD pathology including amyloid-beta, neuropil and 'tau' tangle accumulations (Price and Morris, 1999), and is associated with a major symptom of AD; episodic memory impairment (Lind et al., 2006). Previous large studies of *APOE* ε/*TOMM40* 523 (and hippocampal volume) in older adults are critiqued.

Chapter six examines *APOE* ε/*TOMM40* 523 variations in terms of brain markers of cerebrovascular burden; white matter lesions and brain microbleeds. There is evidence that the presence of cardiovascular or more specifically cerebrovascular disease pathology can increase the future risk of AD and cognitive decline (Debette and Markus, 2010), perhaps through promoting accumulations of amyloid-beta, and/or by increasing vulnerability of the brain to such pathology (Brickmann et al., 2011). Previous large studies of *APOE* ε and these phenotypes in older adults are critiqued. No study has examined *TOMM40* 523 and white matter lesions/brain microbleeds.

Chapter seven examines *APOE* ε/*TOMM40* 523 variation in terms of cognitive ageing, on a wide range of phenotypes including executive function, declarative memory and information processing speed. Based on Chapters 4-6, three-way significant raw nominal associations at $P < 0.05$, between genetic/imaging/cognitive variables are examined for mediation formally with bootstrapping (Preacher and Hayes, 2008). Relatively few samples have the requisite data to conduct such analyses, and Chapter 7 is therefore rare in this regard.

Chapter eight provides an overview of the PhD thesis; it integrates the findings with the literature, highlights limitations to the approach described here, and suggests directions for future studies, using the current sample and in independent samples.

Chapter 2: Methodology

2.1. Recap and overview

The *APOE* gene locus is a significant risk factor for Alzheimer's disease (AD) diagnosis, and worse cognitive ageing (Corder et al., 1994; Deary et al., 2004). Specifically, the ε4 allele is purportedly the 'risk' variant, compared with the neutral ε3 allele and possibly protective ε2 allele (Wisdom et al., 2011; Eisenberg et al., 2010).

There is heterogeneity in associations between *APOE* genotype and different brain phenotypes (e.g. cognitive ability), which suggests that other variables – including genetic – have a modifying or independent effect. The rs10524523 locus (hereafter '*523*') in *TOMM40* is characterised by a variable number of T residues (poly-T repeats; Lutz et al., 2010). The *TOMM40* 523 poly-T repeat genotype was identified by Roses et al. (2010) as having a significant effect on age of AD onset in a small sample of older adults diagnosed with AD (n = 34, mean age = 69.3 years, standard deviation [SD] = 8.3). In individuals with the ε3/ε4 genotype, longer poly-T repeat lengths (≥27) were associated with an earlier age of onset (70.5 years vs. 77.6, $P = 0.02$). Because *APOE* genotype was constant, it could be said that the effect of *TOMM40* 523 was biologically independent; i.e. that any significant effect cannot be attributed to variation in *APOE* genotype. Poly-T repeat lengths are commonly grouped into 'Short' (<20; 'S'), 'Long' (20-29; 'L'), and 'Very-Long' (≥30; 'VL'; Lutz et al., 2010; Linnertz et al., 2012).

As described in Chapter 1 ('*Introduction'*), this thesis aims to investigate the independent effects of the *APOE* ε4 and *TOMM40* 523 gene loci on structural brain MRI (Chapters 4-7) and cognitive ageing (Chapter 7) phenotypes in an existing dataset, the Lothian Birth Cohort 1936 (LBC1936). Here, the sample recruitment as well as cognitive, brain imaging and genotypic assessment methodologies are described, followed by a description of the analytic approach taken. Relevant statistics for the LBC1936 dataset are described in Table 2.1 (e.g. mean ages in years, allele frequencies), and the final analytic

strategy is detailed in Table 2.2. This chapter provides the core methodology and analytic strategy for Chapters 4-7 and most of Chapter 3, although there are study-specific methodological details provided in each specific chapter. Chapter 3 is a proof-of-concept study for subsequent Chapters 4-7 that uses the same LBC1936 cognitive and brain MRI data from around age 73, but investigates a different genetic locus. All of the relevant genetic/analytic information for that study is therefore reported in Chapter 3.

2.2. Dataset used in the present thesis

2.2.1. Ethical statement

Ethics permission for the study protocol was obtained from the Multi-Centre Research Ethics Committee for Scotland (MREC/01/0/56) and from the Lothian Research Ethics Committee (LREC/2003/2/29), in accordance with the Declaration of Helsinki, and all participants gave written, informed consent.

2.2.2. The Lothian Birth Cohort 1936 (LBC1936)

The LBC1936 is a sample of 1091 generally healthy, community-dwelling and non-demented older adults, 1028 of whom completed a test of intelligence at around age 11 years. All of the LBC1936 participants were born in 1936 and most resided in the Edinburgh (Lothian) area of Scotland when recruited for a range of cognitive, physical and sociodemographic assessments in older adulthood. The recruitment and assessment of this sample is described in the following sections, and in two cohort papers by Deary et al. (2007; 2012) and Wardlaw et al. (2011).

2.2.3. Sample recruitment

On June 4th as part of the Scottish Mental Survey 1947 (SMS1947), all children attending school in Scotland that day that were born in 1936 (hence around 11 years old) completed an assessment of intelligence called the Moray House Test no. 12 (simply 'MHT'); a paper-and-pencil test including a variety of reasoning, visuospatial and mathematical questions. This test was validated against the Terman-Merrill revision of the Binet scales, and 70,805 children completed the test (Deary et al., 2007; Scottish Council for Research in Education, 1949).

In 2003, individuals from the Lothian area that were born in 1936 and may have completed the SMS1947 were identified. Upon receiving ethical approval (above), participants born in 1936 were identified from Community Health Index (CHI) numbers held by the Lothian Health Board, providing date of birth and contact details. Potentially eligible individuals (3686) were mailed information on the research project and the nature of participation, with a reply slip stating interest and confirming initial eligibility. This was supplemented with adverts in relevant media seeking participants who may have been assessed on June 4th. Excluding individuals medically unable to participate or otherwise ineligible, this yielded 1226 participants. Participants who later withdrew, could not be contacted or could not undergo assessment before the end of testing were excluded, resulting in 1091 individuals (548 male) that made up the LBC1936. This process is detailed in Figure 2.1, taken from Deary et al. (2007).

2.2.4. Assessment in older age

In the first wave of the LBC1936 study ('Wave 1'), at around age 70 years, participants were retested on the MHT in addition to other detailed cognitive, genotypic, sociodemographic, and physical assessments (N = 1091) at the Wellcome Trust Clinical Research Facility (WTCRF; Western General Hospital, Edinburgh). The sample was generally healthy,

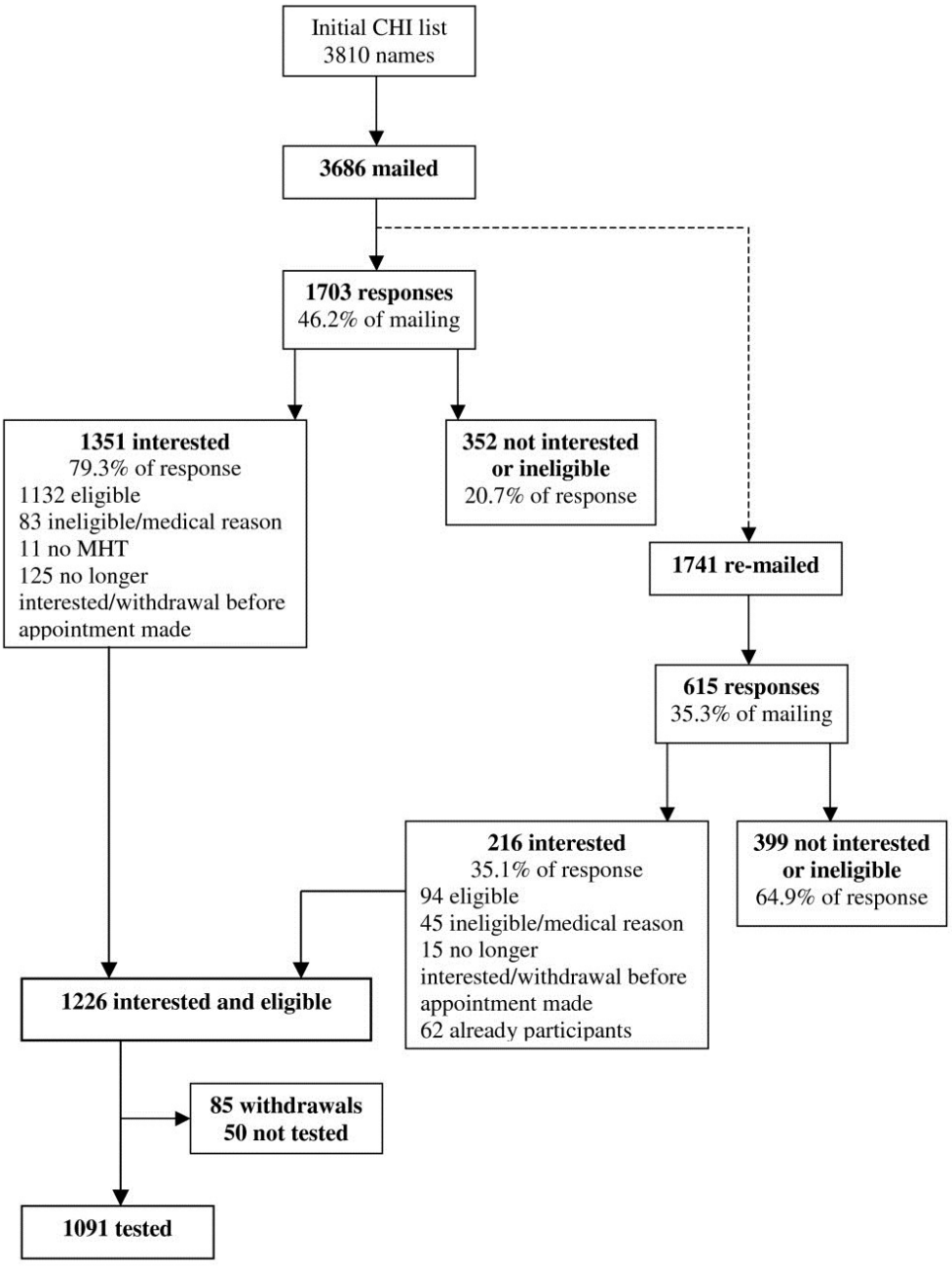
community-dwelling and non-demented. Around three years later, 866 members of the cohort returned for re-testing in the second wave of the study ('Wave 2'). The main reasons for attrition were death, chronic incapacity and general withdrawal (Deary et al., 2012). At this point, in addition to repeating the Wave 1 assessments, the majority of participants also underwent detailed structural brain MRI, on the same day or around the same time, at the Brain Research Imaging Centre (BRIC; also Western General Hospital, Edinburgh).

At both waves, participants were screened for cognitive impairment with the Mini Mental State Examination (MMSE; Folstein et al., 1975), with scores under 24 used to indicate possible dementia. Medical history was elicited via interview, including high blood pressure or hypertension, diabetes, stroke, hypercholesterolemia, and history of any other cardiovascular disease (e.g. heart attack).

2.2.5. Current sample

Of the 1091 total LBC1936 participants that attended Wave 1, 866 attended Wave 2 also, where they underwent repeat cognitive/physical assessments in addition to brain MRI; it is this latter dataset that is primarily examined in this thesis (age ~73 years; Wave 2). Individuals who reported dementia (n = 1), who had MMSE scores below 24 (n = 7; possibly indicative of dementia; Folstein et al., 1975), or did not complete the MMSE at Wave 2 (n = 1) were excluded. Overall, this left 857 participants, of which 811 and 823 participants had successful genotyping for *APOE* and *TOMM40*, respectively. Note that the specific numbers of participants examined in each chapter vary slightly because not every participant had every cognitive and brain imaging phenotype successfully assessed, segmented or processed (Deary et al., 2007; Wardlaw et al., 2011).

Figure 2.1. Flowchart of recruitment for LBC1936 up to around age 70 years ('Wave 1'). Note that this thesis primarily uses data from around age 73 ('Wave 2'; n = 866). Taken from Deary et al. (2007).



2.3. Genotyping

The *APOE* ε haplotype (commonly known as 'genotype') is composed of two single nucleotide polymorphisms (SNPs); rs429358, which causes a Cys130Arg substitution; and rs7412, which causes an Arg176Cys substitution (http://www.ncbi.nlm.nih.gov/gene/348). Different combinations of the rs329358/rs7412 SNPs form the ε2 (Cys/Cys respectively), ε3 (Cys/Arg) and ε4 (Arg/Arg) genotypes (Ringman and Cummings, 2009).

Genotyping for *APOE* was completed at the Wellcome Trust Clinical Research Facility Genetics Core, Western General Hospital, Edinburgh. For *APOE*, DNA was isolated from whole blood, and the target sequences for the each of the rs7412 and rs429358 single nucleotide polymorphisms (SNPs) were genotyped with TaqMan technology. Genotype frequencies were relatively similar to those reported in independent samples; the ε3 allele is the most common (typical frequency ~78.3%), followed by ε4 (~14.5%) and ε2 (~6.4%), although this can vary by population (Eisenberg et al., 2010). In the Wave 2 sample (N = 811), frequencies were ε3 = 76.9%, ε4 = 15.8% and ε2 = 7.3%, and these were in Hardy-Weinberg equilibrium (P = 0.44; specific genotype frequencies are shown in Table 2.1; online Hardy-Weinberg calculator, 2012).

TOMM40 523 poly-T repeat length was genotyped by the laboratory of Dr. Ornit Chiba-Falek (Duke University). Each genomic sample was amplified by polymerase chain reaction (PCR) using fluorescently labelled forward 5'FAM-TGCTGACCTCAAGCTGTCCTG-3' and reverse 5'-GAGGCTGAGAAGGGAGGATT-3'primers. Genotypes were determined on an ABI 3730 DNA Analyzer, using GeneMapper version 4.0 software (Applied Biosystems, Foster City, California) for fragment analysis by the amplified fragment length polymorphism method (Linnertz et al., 2012).

Roses et al. (2010) plotted histograms showing the distributions of poly-T repeat lengths in different *APOE* genotypes; ε3/ε3, ε3/ε4 and ε4/ε4. The poly-T repeat was strongly

linked with the *APOE* ε haplotype; ε4 is linked to L, with ε3 linked to either S or VL alleles (Roses et al., 2010). As can be seen in Figure 2.2, the ε3/ε3 genotype was associated with a relatively bimodal distribution with mainly 'Short' or 'Very-Long' repeat lengths, whilst the ε4/ε4 has an almost entirely unimodal distribution reflecting its linkage with the 'long' genotype. The rarer ε2 allele appeared to show similar linkage to S or VL alleles in *TOMM40* 523 - as per ε3 - although further research is required (Roses et al., 2010).

The distributions of *TOMM40* 523 poly-T repeat lengths in the whole sample (LBC1936 Wave 2 dataset), and in *APOE* ε3/ε3, and ε3/ε4 genotypes, are displayed in Figure 2.3. Generally, the distributions in the LBC1936 sample are as would be expected on the basis of Roses et al. (2010); a relatively bimodal distribution in the ε3/ε3 genotype for both alleles 1 and 2 (i.e. either Short or Very-Long repeat lengths), and for ε3/ε4, a similar bimodal distribution in Allele 1 (reflecting linkage with *APOE* ε3), and a unimodal distribution for Allele 2 (reflecting linkage with *APOE* ε4; Roses et al., 2010).

The allele frequencies for *TOMM40* 523 were S = 41.0%, L = 15.4%, VL = 43.6% (N = 823; in Hardy-Weinberg equilibrium; $P = 0.06$). These are similar frequencies to those reported in independent laboratories, for example Linnertz et al. (2012) report frequencies of S = 45%, L = 11% and 44% in a sample of 177 Caucasian adults.

Figure 2.2. *TOMM40* 523 poly-T repeat length distributions in *APOE* ε3/ε4, and ε3/ε3 genotypes (taken from Roses et al., 2010).

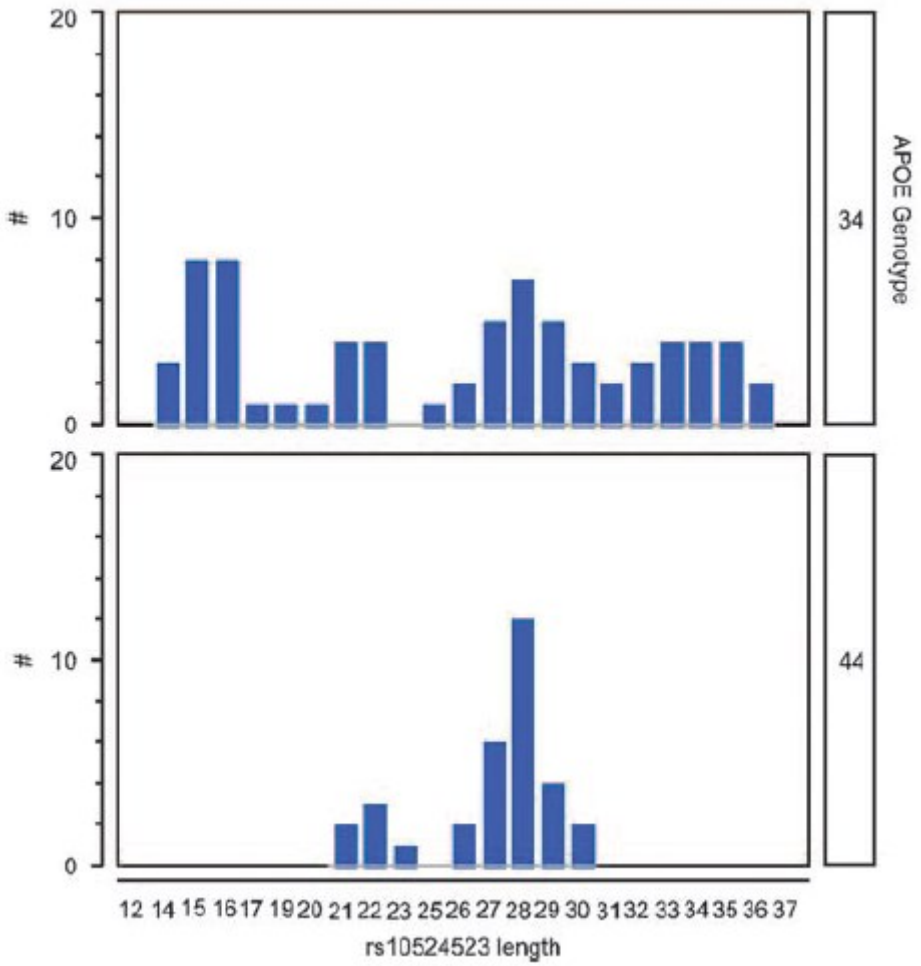
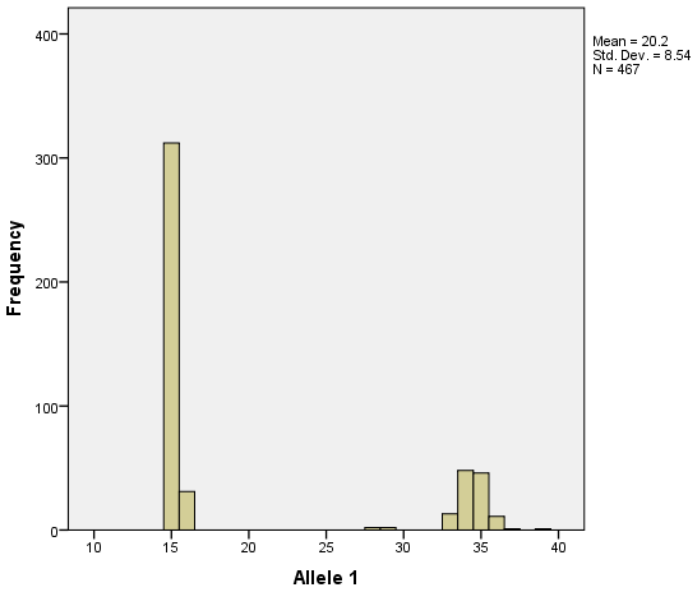
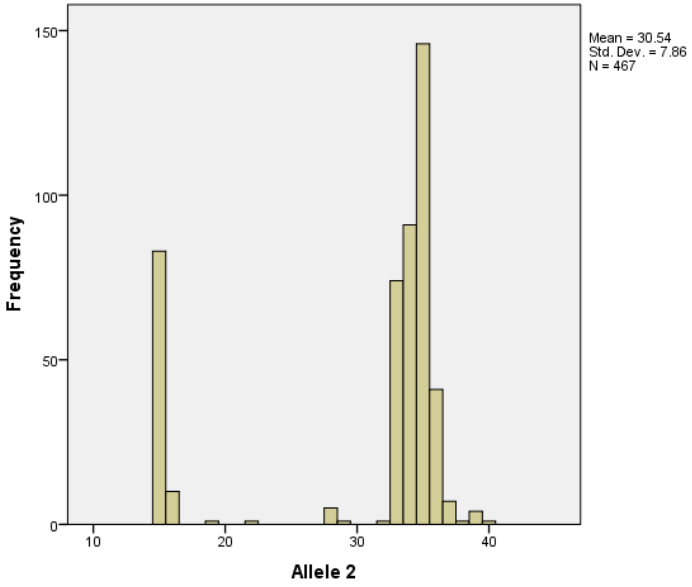


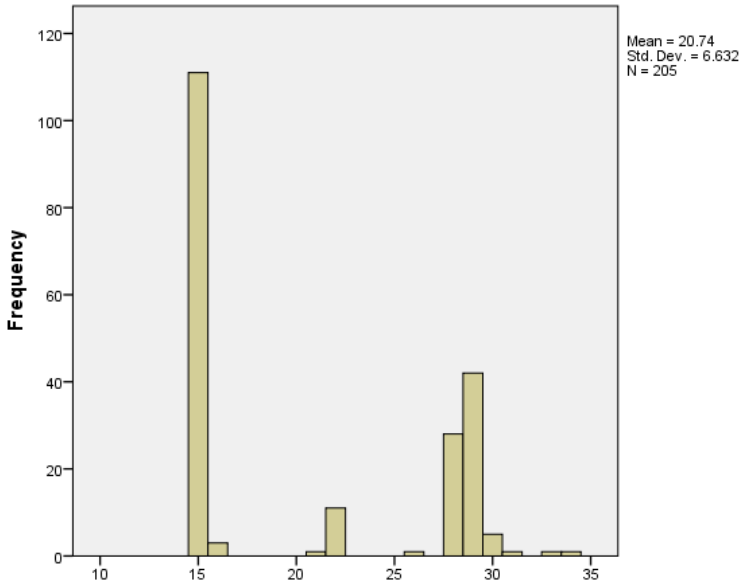
Figure 2.3. *TOMM40* 523 poly-T repeat lengths in a) *APOE* ε3/ε3 (n = 467) and, b) *APOE* ε3/ε4 genotypes (n = 205; note that the central distribution in 'Allele 2' most likely reflects linkage between 'Long' allele and *APOE* ε4 allele; Lutz et al., 2010).

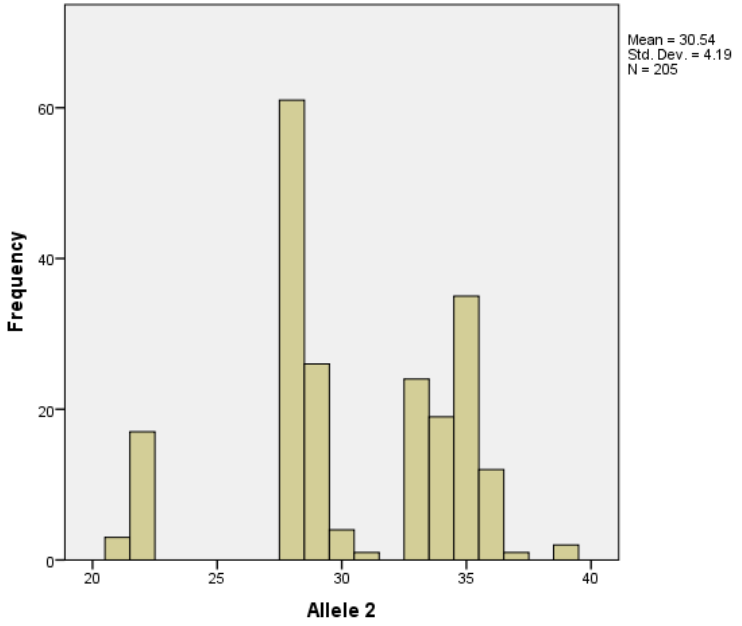
a)





b)





2.4. Brain imaging

The brain MRI protocol is described by Wardlaw et al. (2011), which states that participants underwent whole brain structural MRI, acquired using "a GE Signa Horizon 1.5 T HDxt clinical scanner (General Electric, Milwaukee, USA) equipped with a self-shielding gradient set (33 mT m^{-1} maximum gradient strength) and manufacturer supplied 8-channel phased-array head coil" (pp. 549), lasting around 70 minutes (N = 700 completed scans; mean interval between WTCRF and MRI scan appointments = 65.1 days). In addition to standard structural T2-, T2*- and FLAIR-weighted MRI, the imaging protocol included a high-resolution T1-weighted volume sequence acquired in the coronal plane with field-of-view 256 × 256 mm, imaging matrix 192 × 192 (zero-filled to 256 × 256), 160 1.3mm thick slices giving 1 × 1 × 1.3 mm voxel dimensions (Wardlaw et al., 2011). The repetition, echo and inversion times were 10, 4 and 500 milliseconds respectively. Participants also underwent diffusion tensor and magnetization transfer sequences (Wardlaw et al., 2011).

The specific protocol and methodologies for each imaging phenotype used in subsequent chapters (white matter tract integrity; hippocampal volumes; white matter lesions/brain microbleeds) are briefly outlined in each chapter and detailed in the protocol paper by Wardlaw et al. (2011). Image processing for each of these phenotypes (e.g. segmentation with FSL tools) was completed by staff at BRIC (Wardlaw et al., 2011).

2.5. Cognitive assessment

2.5.1. Moray House Test no.12 (age 11)

This was completed in 1947 at a mean age of 11 years. This assessment has a 45-minute time limit and includes 76 questions with a predominance of verbal reasoning items, with some numerical and visuospatial items also included (Deary et al. 2007). MHT scores were adjusted for age in days at time of assessment, and standardised to an IQ score with a mean of 100 and a standard deviation of 15 for the LBC1936 sample.

2.5.2. Age 73 cognitive assessments

Participants completed a broad battery of cognitive assessments including fluid-type tasks that may be more sensitive to the effects of age compared with more crystal intelligence-type tasks such as the National Adult Reading Test. These tasks assess cognitive ability on a number of domains including working memory, declarative memory, visuospatial ability, executive function and information-processing speed (Deary et al., 2007; 2012). Genetic contributions to age 73 cognitive ability were examined in Chapter 3 and Chapter 7.

Working Memory (Wechsler, 1998a). In the Digit Span Backward task, participants recalled (in reverse order) increasingly long lists of numbers as read by an experimenter. If participants recited the sequence in the correct reverse order, a positive score was given. The length of each digit list increases by one after every second sequence, recited at a regular rate by the experimenter. The first sequence is two digits, increasing ultimately to eight. If two consecutive failures occurred during the same sequence length, the test was discontinued. Digit Span Backward scores were out of a maximum 14.

For the Letter-Number Sequencing task, participants listened to sequences of letters and numbers, as recited by the experimenter. Participants then repeated the sequence to the experimenter, first reciting the numbers in numerical order, and then the letters in alphabetical order. An accurate response for each sequence was required for a positive score, with a maximum score of 21. These sequences became increasingly long, starting with one number and one letter, increasing by one of each (in turn) until the final sequence of four numbers and four letters. Both tasks were taken from the Wechsler Adult Intelligence Test-III ^{UK} (WAIS-III ^{UK}; Wechsler, 1998a).

Processing speed (Wechsler, 1998a). In the Digit Symbol Coding task, participants were presented with a single piece of paper from the WAIS-III ^{UK}. At the top of the page were small reference boxes containing symbols with corresponding numbers beneath them. Below this were several lines of numbers, spanning the page with empty boxes beneath them. With the reference still visible at the top of the page participants were asked to correctly draw as many corresponding symbols as possible within two minutes (Wechsler, 1998a). Participants could score a maximum of 133 on this task, with one point for each accurate symbol drawn.

The Symbol Search task from the WAIS-III^{UK} required participants to determine if a row of symbols on the right-hand side contained one of a pair of targets presented to the left-hand side. Participants indicated this by ticking one of the relevant "yes" or "no" boxes. Participants have two minutes to complete as many trials as possible, with a maximum score of 60. Positive scores were given for accurate identification or rejection of each target symbol on each row (Wechsler, 1998a).

The Simple and Choice-Reaction time (RT) tests provided measures of information processing speed via a self-contained device (Cox et al., 1993). Participants were presented with a rectangular box with a screen, and five numbered response keys numbered left to right

as 1-2-0-3-4. In the Simple RT condition, participants were asked to rest a finger over the 0 button, pressing this button as quickly as possible whenever a zero appeared on the screen. Participants received 8 practice and 20 test trials. In the Choice-RT condition participants placed the second and third fingers of their left and right hands on numbers 1-2 and 3-4 respectively. After each number appeared on the screen, participants pressed the corresponding key as quickly as possible. Each number appeared 10 times in random order. Mean reaction times (in seconds) for correct trials were included. In both Simple and Choice-RT conditions, a one to three second interval occurred between a response and presentation of the next trial (Deary et al., 2007).

The Inspection Time task assessed speed of basic visual processing. Participants were asked to discriminate which of two vertical parallel lines was longer, and were given no time pressure. Participants completed this task on an E-prime programme, with stimulus lines of 5cm and 2.5 cm at 1.6 mm length. Participants completed ten trials at 15 durations from 6 to 200 milliseconds inter-trial interval. Participants indicated which of the two lines were longer by pressing "1" or "2" on a keyboard. The number of correct trials rather was used to score this task (Deary et al., 2007).

Memory (Wechsler, 1998b). The logical Memory (I and II) tests assessed verbal declarative recall for two prose stories at immediate and delayed (around 30 minutes) intervals. Participants were told a short story and asked to recall as much specific content as possible. This was scored by the experimenter for content-specific accuracy as well as less specific thematic recall. This process was then repeated with a second story. Participants were warned they will be asked to recall as much as possible of the two stories at 30 minutes delay. After the delay, recall was again scored for specific and thematic accuracy. Logical memory stories contained 25 elements, with one point for each specific accurate detail.

During the Verbal Paired Associates (I and II) memory task, participants were read a list of unrelated two-word pairs. Participants were then read the first word of each pair and attempted to recall the corresponding paired word. This occurred four times with lists of eight pairs presented, yielding an overall score of 32. At 30 minutes delay, participants attempted recall of each pair. Both tasks were taken from the Wechsler Memory Scale-III ^{UK} (WMS-III^{UK}; Wechsler, 1998b).

Visuospatial ability (Weschsler, 1998a). The Block Design subtask from the WAIS-III^{UK} assessed visuospatial ability. Individuals were asked to reconstruct pictorial designs from three-dimensional blocks placed in front of them, with two minutes permitted for each trial. For the first two trials, participants are given either zero, one or two points depending on whether they complete the task on the first or second trial (or not at all). Thereafter participants were given up to 7 points for each accurate reconstruction, depending on the time to completion. Block Design scores could reach a maximum of 68. This task was taken from the WAIS- III^{UK} (Wechsler, 1998a).

The Spatial Span subtest assessed non-verbal working memory. The examiner tapped increasingly long sequences onto small cube blocks. The participant was asked to re-tap each sequence afterwards. This task was then repeated with participants retapping sequences in reverse order. For each task, a positive score was given if tapping was repeated accurately, with a maximum of 16 for both forwards and backwards yielding an overall total of 32. Spatial Span was taken from the WMS-III^{UK} (Wechsler, 1998a).

Abstract Reasoning (Wechsler, 1998a). The Matrix Reasoning subtest of the WAIS-III (Wechsler, 1998a) displays patterns arranged in a matrix with one section missing and five options available. After five practice trials, participants attempted to identify the underlying rule relating the other three pieces and used this to determine what the missing section should

be. A positive score was given for identification of each correct missing pattern. Participants could score a maximum of 26 on this task.

2.5.3. Age 73 general cognitive factors

Data reduction was applied to the cognitive test scores using principal components analyses (PCA) which produced the following summary cognitive variables. First, 'general intelligence' (*g*) included the six non-verbal Wechsler subtests of Digit Span Backwards, Matrix Reasoning, Letter-Number Sequencing, Block Design, Symbol Search and Digit Symbol Coding. Second, 'processing speed' (g_{Speed}) included Symbol Search, Digit Symbol Coding, Simple RT, Four-Choice RT and Inspection Time. The derivation of these was described by Luciano et al. (2009a). Third, a 'memory' factor (g_{Memory}) was formed from Logical Memory, Spatial Span, Verbal Paired Associates, Letter-Number Sequencing and Digit Span Backwards. The derivation of this variable was described by Houlihan et al. (2010). Strictly speaking PCA does not generate latent underlying 'factors', but this usage is common and is adopted here. In each case, analysis tested for the existence of a large first unrotated component that could be used to form scores for the three variables. Inspection of eigenvalues and scree-plots suggested that a single component should be extracted from the data in each of the three analyses. The first unrotated principal component scores in each domain accounted for 50-54% of the variance for *g*, g_{Speed}, and g_{Memory}, and all individual test scores had moderate to high loadings on their respective first unrotated principal components.

2.6. Demographic information

Genotype frequencies, mean/standard deviation ages in years at time of assessment at the WTCRF, MMSE scores, self-reported cardiovascular disease history prevalence rates and age 11 IQ age data (means and standard deviations) are displayed in Table 2.1, based on the whole sample of 866 participants that completed cognitive testing around the age of 73 years.

Significant differences between genotypes in terms of demographic statistics (outlined in Table 2.1) were tested for with one-way analysis of variance (ANOVA). A significant effect of *APOE* genotype was found for prevalence of self-reported high cholesterol ($F [5, 805] = 2.38$, $P = 0.037$) due to significantly higher prevalence rates in the ε2/ε3 genotype (vs. ε2/ε4 and vs. ε3/ε4; $P < 0.05$), and age at time of testing at the WTCRF ($F [5, 805] = 2.89$, $P = 0.014$) due to significant differences for the ε2/ε2 genotype vs. all others ($P < 0.05$). A significant effect of *TOMM40* 523 genotype was found on prevalence of self-reported high blood pressure ($F [5, 817] = 2.27$, $P = 0.046$), due to significantly higher prevalence rates in S/S genotype vs. L/L, and S/L vs. VL/VL (both comparisons $P <0.05$). No other effects of genotype were found for the remaining variables.

Table 2.1. Descriptive age data (mean/standard deviation; SD) for the Lothian Birth Cohort 1936 ('Wave 2').

	APOE genotype					
	ε2/ε2	ε2/ε3	ε2/ε4	ε3/ε3	ε3/ε4	ε4/ε4
N	3	95	18	472	208	15
Gender; N that are female (% of total)	2	47	10	233	97	6
	(66.7)	(49.5)	(55.6)	(49.5)	(46.6)	(40.0)
Age in years (SD)	73.68	72.62	72.46	72.45	72.49	72.31
	(0.30)	(0.71)	(0.85)	(0.69)	(0.72)	(0.82)
Age 11 IQ (SD)	94.14	99.26	96.74	101.26	102.08	104.04
	(26.43)	(15.86)	(18.94)	(14.21)	(13.40)	(16.16)
MMSE score (SD)	27.77	28.61	28.39	28.82	27.67	28.80
	(3.22)	(1.49)	(1.58)	(1.29)	(0.74)	(1.42)
History of high blood pressure, N (% of total)	3	42	8	241	103	4
	(100)	(44.2)	(44.4)	(51.1)	(49.5)	(26.7)
History of diabetes , N (% of total)	3	10	2	55	19	1
	(100)	(10.5)	(11.1)	(11.7)	(9.1)	(6.7)
History of high cholesterol , N (% of total)	3	26	9	194	97	5
	(66.7)	(27.4)	(50.0)	(41.1)	(46.6)	(33.3)
History of other cardiovascular pathology, N (% of total)	3	26	4	135	64	5
	(100)	(27.4)	(22.2)	(28.6)	(30.8)	(33.3)
History of Stroke, N (% of total)	3	1	1	39	9	2
	(100)	(1.1)	(5.6)	(8.3)	(4.3)	(13.3)
	TOMM40 '523' poly-T repeat genotype					
	S/S	S/L	S/VL	L/L	L/VL	VL/VL
N	125	123	302	18	95	160
Gender; N that are female (% of total)	58	59	157	8	47	75
	(46.4)	(48.0)	(52.0)	(44.4)	(49.5)	(46.9)
Age in years (SD)	72.47	72.45	72.51	72.15	72.54	72.45
	(0.73)	(0.72)	(0.71)	(0.80)	(0.76)	(0.66)
Age 11 IQ (SD)	100.45	101.92	101.38	105.00	101.21	101.38
	(15.20)	(13.79)	(14.12)	(15.09)	(14.67)	(14.10)
MMSE score (SD)	28.78	28.85	28.79	28.94	28.82	28.81
	(1.37)	(1.24)	(1.24)	(1.39)	(1.35)	(1.41)
History of high blood pressure, N (% of total)	64	53	145	4	48	91
	(51.2)	(43.1)	(48.0)	(22.2)	(50.5)	(56.9)
History of diabetes, N (% of total)	8	14	41	1	5	15
	(6.4)	(11.4)	(13.6)	(5.6)	(5.3)	(9.4)
History of high cholesterol , N (% of total)	47	56	124	5	40	64
	(37.6)	(45.5)	(41.1)	(27.8)	(42.1)	(40.0)
History of other cardiovascular pathology, N (% of total)	37	33	83	6	31	45
	(29.6)	(26.8)	(27.5)	(33.3)	(32.6)	(28.1)
History of Stroke, N (% of total)	4	3	27	1	5	11
	(3.2)	(2.4)	(8.9)	(5.6)	(5.3)	(6.9)

Note. SD = standard deviation, *TOMM40* 523 'S' = 'Short' allele, 'L*' = pooled 'Long' and 'Very-long' alleles. These data are based on the sample once people with reported dementia, MMSE scores ≤24 or missing at Wave 2 are removed; detailed in the text body. *APOE* total n = 811, *TOMM40* total n = 823.

2.7. Analytic strategy: *APOE* ε

The *APOE* ε locus includes the *ε*4 allele (generally accepted as the 'risk' variant), *ε*2 (possibly 'protective', but not consistently) and neutral *ε*3 allele. Each person has any two alleles, resulting in six possible combinations (ε2/ε2; ε2/ε3; ε3/ε3; ε3/ε4; ε2/ε4; ε4/ε4). There are a number of ways of analysing this genetic locus, statistically. The final strategy used in this thesis for Chapters 4-7 is detailed in Table 2.2. Tests of significant differences were tested with general linear models (GLM; analysis of variance or simply ANOVA).

2.7.1. Presence vs. absence of the ε4 allele ('Step 1')

Studies of *APOE* ε commonly test the effects of ε4 'risk' allele presence versus absence (i.e. ε2/ε2; ε2/ε3; ε3/ε3 vs. ε3/ε4; ε2/ε4; ε4/ε4; Wisdom et al., 2011; Deary et al., 2004). Frequency of the ε4/ε4 genotype is often low, and pooling ε4/ε4, ε3/ε4 and ε2/ε4 participants can improve power (Luciano et al., 2009a).

Some studies exclude the ε2/ε4 genotype from analysis because it includes 'protective' and 'risk' alleles which could potentially cancel one another out and reduce power of the ε4 presence effect (Wisdom et al., 2011). Here, analyses included the ε2/ε4 genotype because:

1) The suggested protective effect of the ε2 allele is much less consistent, compared with the deleterious effect of the ε4 allele (Ashford, 2002; McCarron et al., 1999). For example, in a large meta-analysis of 20 independent Caucasian samples (N = 4, 946), Bertram et al. (2007) reported a significant deleterious effect of ε3/ε4 (vs. ε3/ε3; odds ratio [OR] = 2.7, 95% C.I.'s = 2.2-3.2) and ε4/ε4 (OR = 12.5, 95% C.I.'s = 8.8-17.7), but no effect of ε2/ε3 (OR = 0.9, 95% C.I.'s = 0.4-2.5), statistically adjusted for age and study centre.

2) The *APOE* ε2 allele has occasionally been associated with deleterious effects. For example, Berlau et al. (2009) examined neuropathology in 85 very-older adults that had died (mean age = 97.3, SD not provided) in terms of Braak plaque staging (reflecting the degree of neurofibrillary burden including amyloid plaque and 'Tau' tangles; detailed in Chapter five; Braak and Braak, 1991). They reported that ε2 carriers showed significantly increased Braak plaque staging (OR = 8.2, 95% C.I.'s = 2.2-30.4), increased neuritic plaques (OR = 4.4, 95% C.I.'s = 1.3-15.2; vs. ε3/ε3 genotype). The ε4 and ε2 alleles showed similarly deleterious effects, therefore (see also Trachtenberg et al., 2012; Farrer et al., 1997).

2.7.2. APOE ε single point mutation test: ε3/ε4 vs. ε3/ε3 ('Step 2')

The *APOE* ε4 allele may have dose-response deleterious effects where the ε4/ε4 genotype is more strongly associated with brain imaging or cognitive phenotypes, compared with ε2/ε4 or ε3/ε4 (e.g. Bertram et al., above). The ε4/ε4 genotype is rare, however, which makes analysis of this locus less reliable (Eisenberg et al., 2010; Hattersley and McCarthy, 2005). To account for this, studies have previously tested for a significant effect of ε3/ε4 vs. 'neutral' ε3/ε3 (Deary et al., 2004; Wisdom et al., 2011; See Table 2.2).

2.7.3. Presence vs. absence of the ε2 allele ('Step 3')

Although the ε2 allele 'protective' effect is less consistent compared with the ε4 'risk' effect, independent studies have previously tested for the effects of ε2 allele presence vs. the neutral genotype (ε2/ε2; ε2/ε3; vs. ε3/ε3; Wisdom et al., 2011).

2.7.4. Summary

The *APOE* ε genotype is composed of any two of the ε2 (protective), ε3 (neutral) and ε4 (risk) alleles. The first analytic step tested the effects of *APOE* ε4 allele presence vs. absence, i.e. pooled ε2/ε4, ε3/ε4 and ε4/ε4 genotypes versus pooled ε2/ε2, ε2/ε3 and ε3/ε3 (*'Step 1'*). The second analytic step tested the effect of a single point mutation and removed carriers of the 'protective' ε2 allele: ε3/ε4 vs. ε3/ε3 genotypes only (*'Step 2'*; Deary et al., 2004). The third and final analytic step tested genotypes which may be protective for neurodegenerative pathology, compared with the neutral genotype; i.e. pooled ε2/ε3 and ε2/ε2 versus ε3/ε3 (Deary et al., 2004; Luciano et al., 2009a; *'Step 3'*). This is detailed in Table 2.2.

2.8. Analytic strategy: *TOMM40* '523' poly-T repeat genotype

The *TOMM40* 523 genetic locus includes the 'S', 'L', and 'VL' alleles. There are a number of ways of analysing this genetic locus, statistically. The final strategy used in this thesis for Chapters 4-7 is detailed in Table 2.2.

On the basis of findings by Roses et al. (2010; see Figure 2.2), studies that investigate the *TOMM40* 523 locus usually group repeat lengths into categories, to improve power; namely 'Short; S' (<20 T residues), 'Long; L' (≥ 20) and 'Very-long; VL (≥ 30) (Lutz et al., 2011; Linnertz et al., 2012). This results in six different genotypes (S/S; S/L; S/VL; L/VL; VL/VL). In the first analytic step, in the whole sample, a GLM tested for a significant effect of *TOMM40* 523 genotype (i.e. S/S; S/L; L/L; L/VL; VL/VL; *'Step 1'*), followed by post-hoc contrasts if a main effect is found (i.e. $P < 0.05$).

To investigate the effects of *TOMM40* 523 repeat length independent of biological variation in *APOE* genotype, analysis then focussed separately on two different *APOE* ε genotype subgroups; firstly participants with the ε3/ε4 genotype (*'Step 2'*; e.g. Roses et al,.

2010). Devi et al. (2012) reported that this genotype had the highest accumulation of amyloid precursor protein (APP) in AD brain mitochondria (vs. ε3/ε3 and ε4/ε4 genotypes; assessed by immunoblot analysis), and this correlated strongly with two indicators of mitochondrial dysfunction (cytochrome C oxidase activity and reactive oxygen species hydrogen peroxide; 'H_2O_2'). In terms of a possible interaction between APP accumulation and mitochondria translocase processes, it is not known if the *TOMM40* 523 genotype may offset or interact biologically with the ε3/ε4 genotype (Devi et al., 2012). Finally, analysis focussed on participants with the neutral *APOE* genotype (ε3/ε3) *('Step 3')*, because this eliminates variance associated with putatively protective and risk *APOE* alleles (Johnson et al., 2011; Roses et al., 2010).

In large sample of Caucasians, linkage between the *APOE* ε genotype and *TOMM40* 523 length (i.e. ε4 links primarily to 'L', ε3 primarily to 'S' or 'VL') is such that in the *APOE* ε3/ε3 genotype, relatively few L carriers would be predicted whereas in the ε3/ε4 genotype typically one L allele would be predicted in addition to either an S or VL allele. Slight errors in poly-T repeat length measurement may occur through PCR 'slippage' and this may result in repeat lengths that are close to the L/VL boundary being incorrectly classified (Linnertz et al., 2012). To attempt to control for this, in Steps 2 and 3 the L and VL alleles were pooled into an 'L*' group; participants with the S/S genotype were compared with those carrying only one S allele (pooled S/L and S/VL; hereinafter S/L*), and also against participants carrying no S alleles (pooled L/L, L/VL and VL/VL; hereinafter L*/L*). This thesis therefore tested for significant effects of *TOMM40* 523 genotype (S/S; S/L*; L*/L*) on cognitive/imaging variables in Steps 2 and 3 (*APOE* ε3/ε4 and ε3/ε3 subgroups respectively). The analytic strategy for *TOMM40* 523 is described in Table 2.2.

2.9. Covariate models

Three statistical models were tested to investigate the effects of the *APOE* ε and *TOMM40 523* loci on cognitive/brain imaging variables. First, all models controlled for gender and age in days at neuroimaging to account for general sex-differences in brain morphology, and the huge confounding effect that increasing age can have on brain-related and fluid-type cognitive variables; ('Model 1'; Wardlaw et al., 2011; Hofer and Sliwinski, 2001).

Second, statistically significant effects ($P < 0.05$) were re-tested additionally controlling for age 11 IQ ('Model 2'). This allows the current study to partly account for differences in childhood intelligence and its subsequent possible influence on health-related variables throughout the lifespan, such as disease management (Batty et al., 2007). Finally, significant associations that survived correction for age 11 intelligence were then re-tested additionally controlling for the following covariates: self-reported history of high blood pressure/hypertension, stroke, type-2 diabetes, high cholesterol, and other cardiovascular diseases. This reduces the chance that any significant associations occur as secondary to genetic associations with cerebrovascular pathology ('Model 3'; Schiepers et al., 2012; Zade et al., 2010).

Models were additive, meaning that Model 3 included age 11 IQ when significant in Model 2. An additive stepwise approach to modelling was taken because running the final 'age + gender + age 11 IQ + cardiovascular disease history' model alone was considered overly restrictive a-priori.

2.10. Additional analytic details

An online calculator was used to perform tests of Hardy-Weinberg equilibrium and determine minor allele frequencies (http://www.had2know.com/academics/hardy-weinberg-equilibrium-calculator-3-alleles.html). Data were otherwise analysed with the Predictive Analytics SoftWare (PASW, version 17) statistics programme. Specifically, univariate general linear models (GLMs) tested the effects of separate *APOE* and *TOMM40* genotypes upon cognitive/brain imaging variables. Outliers of more than 3 standard deviations from mean values were removed from age 11 intelligence (N = 7). Outliers were not removed from imaging variables. Where appropriate, to protect against type 1 errors, the false discovery rate (FDR) was used to estimate the number of significant findings controlling for multiple testing (Benjamini and Hochberg, 1995). An Excel program (Pike 2011) was used to conduct classical one-stage FDR based on associations with white matter integrity. All *P* values are raw unless stated as being FDR-adjusted, and *P*-values < 0.05 are considered significant.

2.10.1. Power analyses

Wardlaw et al. (2011) conducted power estimation for the LBC1936 sample using the nQuery Advisor (www.statistical-solutions-software.com). With a sample size of n = 650, they calculated 80% power (at $P < 0.05$) to find: 1) an *r* correlation with a value of 0.11, and 2) a variable that contributed 0.72% explained variance to a model, additional to four prior covariates that explained an initial 40% of the variance. These effect sizes are typical for associations between specific genetic variations (e.g. *APOE*) and brain imaging/cognitive phenotypes (Penke et al., 2010a; Wisdom et al., 2011). The current LBC1936 dataset would therefore seem sufficiently powered to find significant associations in subsequent analyses (Wardlaw et al., 2011).

2.11. Final analytic strategy

The final analytic strategy is displayed in Table 2.2, including *APOE/TOMM40* 523 analysis and stepwise inclusion of covariate models.

Table 2.2. Final analytic strategies for *APOE* ε and *TOMM40* '523' poly-T repeat gene loci for Chapters 4-7.

	APOE ε	*TOMM40 '523'*
Gene locus:	rs7412 + rs429358 (simply 'ε genotype')	Poly-T repeat at rs10524523
Step 1:	ε4 present (vs. ε4 absent)	Overall effect of genotype? (S/S; S/L; VL/L; L/L; VL/VL) (in the whole sample)
Step 2:	ε3/ε4 (vs. ε3/ε3)	S/S; S/L*; L*/L* (in *APOE* ε3/ε4 genotype subgroup)
Step 3:	ε2 present (vs. ε3/ε3)	S/S; S/L*; L*/L* (in *APOE* ε3/ε3 genotype subgroup)
Covariate models:	Model 1: Age + Gender Model 2: Age + Gender + Age 11 IQ Model 3: Age + Gender + Age 11 IQ + Cardiovascular disease history	

Note. The term 'ε4 present' includes participants with ε3/ε4; ε2/ε4; ε4/ε4 genotypes pooled together, while 'ε4 absent' includes all other genotypes. 'ε2' present includes participants with ε2/ε3 and ε3/ε3 genotypes only. *TOMM40* 523 L* = L and VL alleles pooled.

Chapter 3: *ADRB2*, cognitive ageing and brain white matter integrity

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3.1 Abstract

The non-synonymous mutations arg16gly (rs1042713) and gln27glu (rs1042714) in the adrenergic β-2 receptor gene (*ADRB2*) have previously been significantly associated with cognitive function and brain white matter integrity. The current study aimed to replicate these findings and expand them to a broader range of cognitive and brain imaging phenotypes. The sample used is a community-dwelling group of older people, the Lothian Birth Cohort 1936. They had been assessed cognitively at age 11 years, and undertook further cognitive assessments and brain diffusion MRI tractography in older age. The sample size range for cognitive function variables was n = 686 to 765, and for neuroimaging variables was n = 488 to 587. Previously-reported findings with these genetic variants did not replicate in this cohort. Novel, nominally significant associations were observed; notably, the integrity of the left arcuate fasciculus mediated the association between rs1042714 and the Digit Symbol Coding test of information processing speed. No significant associations of cognitive and brain imaging phenotypes with *ADRB2* variants survived correction for false discovery rate. Previous findings may therefore have been subject to type 1 error. Further study into links between *ADRB2*, cognitive function and brain white matter integrity is required. This chapter provides proof of concept for subsequent chapters 4-7, by demonstrating a mediating relationship between genetic, brain imaging and cognitive phenotypes in LBC1936 (based on raw, nominal unadjusted significant associations).

3.2. Introduction

3.2.1. The ADRB2 gene locus

The adrenergic biologic pathway stimulates catecholamine-induced activation of adenylate cyclase, which catalyzes the conversion of adenosine triphosphate (ATP) to 3',-cyclic AMP (cAMP), a secondary messenger that influences cellular replication, differentiation and survival; the adrenergic pathway is therefore important for brain development and function (Kulminski et al., 2010).

The adrenergic β-2 receptor gene (*ADRB2*) is widely expressed in the brain where it plays many roles including signal transduction of adrenaline, noradrenaline and to a lesser extent dopamine (Brodde 2008; Dohlman et al., 1991). Two single nucleotide polymorphisms (SNPs), rs1042713 which causes an Arg16Gly amino acid substitution, and rs1042714 which causes a Gln27Glu amino acid substitution, affect adrenergic receptor sensitivity (Cagliani et al., 2009; Green et al., 1994). Arg16Gly and Gln27Glu polymorphisms have been significantly associated at $P < 0.05$ (hereafter simply 'associated') with increased risk of neurodevelopmental disorder (Cheslack-Postova et al., 2007, N = 331, e.g. odds ratio for rs1042714 Glu/Glu = 1.66, 95% confidence intervals = 1.07–2.58; vs. Gln/Gln) and, separately, with differences in cardiovascular phenotypes such as diastolic blood pressure (Snieder et al., 2002; N = 395, e.g. mean mmHg for rs1042714 Gln/Gln = 56.3 vs. Glu/Glu = 58.0, $r^2 = 2.6\%$, $P < 0.01$).

Bochdanovits et al. (2009) tested rs1042713 and rs1042714 in two Dutch samples of 391 young (mean age 12.7 years) and 409 adult (36.7 years) participants assessed on Dutch adaptations of the Wechsler Adult Intelligence Scale-III^{UK} (WAIS-III^{UK}). For rs1042713, the 16Gly (G allele) was associated with lower performance IQ scores in the young Dutch sample only ($\chi 2$ statistic = 6.42, $P = 0.01$). They also assessed the Lothian Birth Cohort 1936 (LBC1936) which comprised 1063 older adults with relevant data. They were tested on the

Moray House Test No.12 (MHT; a general intelligence-type test) at two ages, namely 11 and 70 years, and the Matrix Reasoning subtask from the standard WAIS-III^{UK} also at age 70 years. Matrix Reasoning was significantly associated with both rs1042713 (standardised β = 0.07; hereafter β; P = 0.025) and rs1042714 (β = 0.08, P = 0.014) positively and additively in the direction of the 16Gly and 27Glu (G) alleles, respectively. The rs1042713 SNP was also associated with age 70 MHT (β = 0.07, P = 0.025), and rs1042714 with age 11 MHT (β = 0.07, P = 0.023).

3.2.2. ADRB2, brain white matter tract integrity and cognitive ability

Diffusion tensor MRI (DTI) and quantitative tractography allow examination of brain white matter microstructure *in vivo* in specific white matter tracts thought to relate to cognitive functions (Behrens et al., 2007; Pierpaoli et al., 1996). DTI measures the magnitude and directional coherence of water molecule diffusion and, because water molecule diffusion is preferentially constrained along the principal fibre direction by axonal membranes and myelin sheaths, this property can be used to assess white matter structural integrity (Behrens et al., 2007; Pierpaoli et al., 1996). Fractional anisotropy (FA) and mean diffusivity (MD) are examples of common DTI-derived metrics, and reflect the magnitude and level of directional coherence of water molecule diffusion (Pierpaoli et al., 1996). Specifically, FA measures are high in healthy, structurally intact, coherently organised white matter, but fall in diseased tissue, with MD measures the inverse (i.e. lower values in intact tissue).

In a subsample consisting of 162 LBC1936 participants who had by that time undergone diffusion MRI tractography as well as cognitive assessments (Penke et al., 2010b), the rs1042714 G allele was significantly associated with reduced FA (β = -0.19, P = 0.008) in the left arcuate fasciculus indicating lower white matter integrity. In the splenium of corpus callosum the G allele was associated with higher FA (β = 0.16) and lower MD (β = -0.17, P =

0.026), indicating greater integrity. This allele was associated with higher age 70 MHT and age 73 Matrix Reasoning performance, both with (β = 0.24, *P* = 0.003 and β = 0.23, *P* = 0.003) and without (β = 0.19, *P* = 0.008 and β = 0.23, *P* = 0.003) controlling for childhood intelligence as assessed by the MHT. These data therefore indicate that variation in the *ADRB2* gene may have an effect on the change in cognitive function between childhood and older age. In addition, Penke et al. (2010b) found that the integrity of the splenium correlated positively with Matrix Reasoning and MHT performance. These neuroimaging and cognitive variables were each associated with the G allele of rs1042714. Penke et al. (2010a) further reported that controlling for splenium integrity attenuated the relationship between rs1042714 and both MHT and Matrix Reasoning adjusted for age 11 cognitive ability, indicating that this tract partially mediated the SNP-cognitive ageing associations (Sobel mediation test *P* values = 0.058 and 0.077). Penke et al. reported relatively large effect sizes (β) for an association study of common SNPs, and it is important to attempt replication in a larger sample.

3.2.3. Current study

Bochdanovits et al. (2009) suggested that the G alleles of rs1042713 and rs1042714 could have been under positive selection for higher cognitive ability in humans, whereas Penke et al. (2010b) noted that the G allele of rs1042714 appears to be protective against age-related cognitive decline (Deary et al., 2009). In the current study it is investigated: i) whether the results of Penke et al. (2010b) can be replicated in the larger, full LBC1936 sample; while ii) extending the number of cognitive domains and white matter tracts tested for association with *ADRB2* SNPs, in further exploratory analyses. By formally testing for a mediating relationship between genetic, brain imaging and cognitive variables in the LBC1936, this chapter is a proof-of-concept study for subsequent Chapters 4-7.

3.3. Methods

3.3.1. Sample and procedure

The recruitment and testing of the LBC1936 sample used here is described in Chapter 2 (‘*Methodology’*; Deary et al., 2007; 2012).

3.3.2. Cognitive assessment

The cognitive tests completed by members of the LBC1936 sample are described in Chapter 2 (‘*Methodology’*), as well as the protocol paper (Deary et al., 2007). Data reduction was applied to the cognitive test scores using principal components analyses (PCA) which produced summary cognitive variables, as detailed in Chapter 2 (‘*Methodology’*). The first unrotated principal component scores in each domain accounted for the variance as follows: 50.1% for *g*, 49.8% for g_{Speed}, and 53.6% for g_{Memory}. All individual test scores had moderate to high loadings on their respective first unrotated principal components for *g* (*r* range = 0.64 to 0.75), g_{Speed} (*r* range = -0.51 to 0.78) and g_{Memory} (*r* range = 0.54 to 0.82).

3.3.3. Childhood intelligence

The Moray House Test no.12 (MHT) completed at around age 11 years is described in Chapter 2 (‘*Methodology’*).

3.3.4. Diffusion MRI and tractography analysis

The core brain MRI procedure is described in Chapter 2 (‘*Methodology’*; see also the protocol paper by Wardlaw et al., 2011).

Diffusion MRI data acquisition and pre-processing with FSL is detailed in the protocol paper by Warldaw et al. (2011). Briefly, FSL was used to generate parametric maps

of water diffusion tensor parameters MD and FA. These biomarkers measure the magnitude and directional coherence of water molecule diffusion *in vivo*, and since water molecules diffuse preferentially along the principal fibre direction, can be used to assess white matter structural integrity (Pierpaoli et al., 1996). Specifically, MD takes low and FA high values in healthy, structurally intact white matter, but rise or fall respectively in diseased tissue. Further details are provided by Wardlaw et al. (2011).

Twelve tracts-of-interest were identified using probabilistic neighbourhood tractography, a novel approach for automatic and reproducible tract segmentation (Clayden et al., 2007), as implemented in the TractoR package for fibre tracking analysis (Clayden et al., 2011; http://www.tractor-mri.org.uk). Tracts assessed were the genu and splenium of corpus callosum, and bilateral anterior thalamic radiations, rostral cingulum bundles, arcuate, uncinate and inferior longitudinal fasciculi. Tract masks generated by this method were overlaid on the MD and FA parametric maps and tract-averaged values of these biomarkers, weighted by the connection probability, determined for each tract in every subject (Wardlaw et al., 2011). In general, probabilistic neighbourhood tractography was able to segment the twelve tracts-of-interest reliably (see Wardlaw et al., 2011 for more details).

PCA was conducted on the tract-averaged water diffusion parameters for these twelve pathways, giving clear single-factor models for FA (g_{FA}) and MD (g_{MD}) that accounted for 38.8% and 39.4% of the overall variance respectively. The ventral cingulum was not included in the PCA because the rostral and ventral cingula are subdivisions of the same tract. All individual tracts had moderate to high loadings on their respective first unrotated principal components (FA r range = 0.33 to 0.70; MD r range = 0.19 to 0.71). This indicates that the integrity of white matter tracts is to a substantial degree shared among different tracts throughout the brain, suggesting potential common causes (Lopez et al., 2012). These general

white matter integrity factors have been found to be associated with cognitive abilities in this sample (Penke et al., 2010a; Penke et al., 2012).

3.3.5. ADRB2 genotyping

ADRB2 SNPs rs1042713 and rs1042714 were genotyped from DNA isolated from whole blood by KBiosciences (http://www.kbioscience.co.uk) using their proprietary genotyping assay, KASPar.

3.4. Statistical analyses

Analysis first attempts to replicate the results reported by Bochdanovits et al. (2009) and Penke et al. (2010b) using the same cognitive measures and covariates. In a second step, exploratory analyses are conducted on a broader range of cognitive measures and white matter tracts to test further associations between *ADRB2* SNPs, neurostructural indicators, and cognitive ability as well as lifetime cognitive change.

All associations were controlled for gender in addition to age in days at time of cognitive and neuroimaging assessment. Penke et al. (2010b) reported that associations that controlled for age and gender were unchanged when cardiovascular disease histories were entered as covariates. Any influence of cardiovascular pathologies such as high blood pressure on genotype-brain imaging/cognitive phenotype associations may have been different in the larger sample used here (n = 866) compared with the smaller sample used by Penke et al. (n = 162). The current study therefore included associated cardiovascular covariates in exploratory analyses as part of the final model in this larger sample unless otherwise specified. To examine the effects of *ADRB2* upon lifetime cognitive change, cognitive task scores from age 73 were controlled for age 11 MHT score.

In the current study, PLINK (Purcell et al., 2007; http://pngu.mgh.harvard.edu/~purcell/plink) provided summary statistics for minor allele frequencies and was used to perform tests of Hardy-Weinberg equilibrium. Haploview provided linkage disequilibrium statistics (Barret et al., 2005; http://www.broadinstitute.org/scientific-community/science/programs/medical-and-population-genetics/haploview/haploview). Data were otherwise analysed with the Predictive Analytics SoftWare (PASW, version 17; http://www-01.ibm.com/software/uk/analytics/spss) statistics programme.

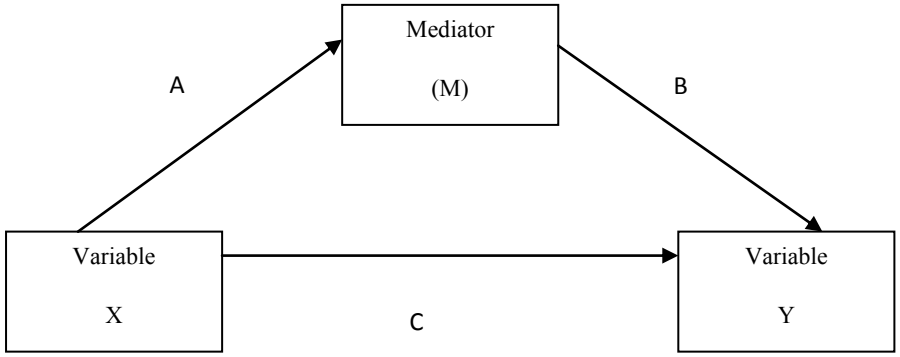
Mediation analysis was used to test the indirect effect of the predictor variable (*ADRB2*) upon the outcome (cognitive function), through the hypothesised mediator (white matter integrity). Mediation analysis was run using the INDIRECT bootstrapping macro (Preacher and Hayes, 2008). In a simple example mediation model (see Figure 3.1), variable X's effects on variable Y can be either direct or indirect via variable M. Path A represents the effect of X on M, while path B represents the effect of M on Y, partialling out the effect of X. The direct effect of X on Y is represented by path C. The indirect effect can then be quantified as the combined product of paths A and B. The bias-corrected bootstrapping point estimate coefficients that are reported here each reflect this indirect product (Preacher and Hayes, 2008). These point estimate coefficients are unstandardised and averaged over 5000 bootstrap estimates (Preacher and Hayes, 2008). Bias-corrected bootstrapping has an advantage over percentile bootstrapping in that it corrects for any skew in the population, including the bias created by the central tendency of the estimate (Efron and Tibshirani, 1993; Fritz and MacKinnon, 2007).

To protect against type 1 error, the false discovery rate (FDR) was used to estimate the number of significant findings controlling for multiple testing (Benjamini and Hochberg,

1995). An Excel macro (Pike, 2011) was used to conduct classical one-stage FDR based on *ADRB2* associations with white matter integrity and cognitive function.

All initially-reported *P*-values are raw (and then FDR-adjustment is applied), β coefficients are standardised (and are equivalent to semi-partial *r* correlations), and *P*-values < 0.05 are considered nominally significant. Linear regression analyses tested the additive effects of *ADRB2* rs1042713 and rs1042714 SNP G alleles upon white matter and cognitive variables. All associations are reported in the direction of these alleles.

Figure 3.1. Schematic showing a standard mediation model, where variable X's effects on variable Y can be either direct or indirect via variable M. Path A represents the effect of X on M, while path B represents the effect of M on Y, partialling out the effect of X. The direct effect of X on Y is represented by path C. The indirect effect can then be quantified as the combined product of paths A and B (Preacher and Hayes, 2008).



3.5. Results

Of the 866 members of the cohort who returned for cognitive testing in Wave 2, participants were excluded if they reported being ambidextrous or left-handed at either wave (n = 57; reflecting asymmetries in white matter tract connectivity that have been associated with handedness; Buchel et al., 2004), had MMSE scores below 24 (n = 7) or not completed at Wave 2 (n = 1), reported diagnosis of dementia (n = 1), or failed/refused genotyping for rs1042713 (n = 34) or rs1042714 (n = 36). One participant was removed from analysis because of a discrepancy between their directly genotyped *ADRB2* data (as described above) and genome wide association study (GWAS) data which were also available for the current sample. Removing this participant did not affect any final results and this chapter reports only directly genotyped data as performed by KBioscience. Cognitive and SNP data were ultimately obtained from 765 (rs1042713) and 762 (rs1042714) individuals, and neuroimaging data were obtained from 650 and 647 subjects, respectively. Outliers of more than 3 standard deviations from mean values were removed from all cognitive variables.

Minor allele frequencies in the sample for rs1042713 were A = 34%, and for rs1042714 G = 48%. Genotype frequencies for rs1042713 were AA = 11%, AG = 46% and GG = 43%, and for rs1042714 GG = 23%, GC = 50% and CC = 27%. These frequencies are relatively similar to those reported in other large independent samples (Snieder et al., 2002). Exact tests performed in PLINK confirmed that rs1042713 (P = 0.52) and rs1042714 (P = 0.91) were individually in Hardy-Weinberg equilibrium. Both SNPs were in high linkage disequilibrium in LBC1936, however (r^2 = 0.47, D' = 1.00, C.I = 0.98-1.00).

3.5.1. Attempted replication of previous findings

Replication of previous results by Bochdanovits et al. (2009) and Penke et al. (2010b) was attempted first. Bochdanovits et al. examined Matrix Reasoning scores at age 70 and MHT scores at ages 11 and 70, while Penke et al. examined MHT at age 70 and Matrix Reasoning at age 73 both adjusted and unadjusted for age 11 cognitive ability. For comparison, Table 3.1 shows association statistics (β coefficients and *P* values) as reported by Bochdanovits et al., and Penke et al. and also when re-tested in the present sample.

In a larger number of the LBC1936 tested at mean age 70 years (n = 1063; Wave 1), Bochdanovits et al. (2009) reported associations between *ADRB2* SNPs and abstract reasoning tasks, controlling for age and gender. When tested again in the current subsample of participants that underwent repeat cognitive testing in Wave 2, there were no significant associations between rs1042713 or rs1042714 with MHT or Matrix Reasoning at age 70, or MHT at age 11 years (all P > 0.05; see Table 3.1). The effect sizes were similar to those found by Bochdanovits et al. but non-significant. The current study included right-handed participants only. To test for this as a source of discrepancy, the original data (Wave 1) of Bochdanovits et al. were re-analysed in right-handed participants only (n = 954). With an otherwise identical analytic strategy, the findings by Bochdanovits et al. remained at or very close to statistical significance except for one association; namely rs1042714 and age 11 MHT (β = 0.05, *P* = 0.165). Another source of discrepancy may be selective attrition of more cognitively impaired individuals from Wave 1 to Wave 2 of testing in old age. Independent samples t-tests showed that in right-handed participants, those who attended both Waves 1 and 2 (n = 866)—compared with those who attended Wave 1 only—had higher scores on age 11 MHT (mean = 50.16, SD = 11.00 vs. mean = 47.62 SD = 10.53, *t* = -2.89, *P* = 0.004, Cohen's *d* = 0.24), age 70 MHT (mean = 65.22, SD = 7.55 vs. mean = 62.80, SD = 8.66, *t* = -

3.59, $P < 0.001$, Cohen's $d = 0.30$) and age 70 Matrix Reasoning (mean = 13.92, SD = 5.03 vs. mean = 12.27, SD = 5.14, $t = -4.15$, $P < 0.001$, Cohen's $d = 0.32$).

The previous pilot study on the subsample of the present cohort that had been brain scanned at that time (n = 162) reported seven significant associations (Penke et al., 2010b), which are shown here in Table 3.1. In the current full sample, all seven associations were markedly reduced in effect size. Only the one between rs1042714 and left arcuate fasciculus FA ($\beta = -0.12$, $P = 0.006$) was still significant and the one between rs1042714 and age 73 Matrix Reasoning ($\beta = 0.07$, $P = 0.053$) showed a statistical trend. This trend attenuated when age 11 MHT score was added as a covariate ($\beta = 0.05$, $P = 0.116$). Because there were no significant associations with splenium of corpus callosum FA or MD, replicating the mediation reported by Penke et al. was not attempted.

Table 3.1. Linear *ADRB2* SNP associations with cognitive function in two previous reports compared with the present sample.

				Present sample (n = ~765)
Previous report	SNP (G alleles)	Cognitive task	β (p)	β (p)
Bochdanovits et al., (2009; n = 1063)	rs1042713	Matrix Reasoning (age 70)	0.07 (0.020)	0.05 (0.154)
		Moray House Test (age 70)	0.07 (0.025)	0.04 (0.240)
	rs1042714	Matrix Reasoning (age 70)	0.08 (0.014)	0.06 (0.124)
		Moray House Test (age 11)	0.07 (0.023)	0.06 (0.109)
Penke et al. (2010b; n = 162)	rs1042714	Moray House Test (age 70)	0.24 (0.003)	0.03 (0.400)
		Adjusted for age 11 IQ	0.19 (0.008)	0.00 (0.982)
		Matrix Reasoning (age 73)	0.23 (0.003)	0.07 (0.053)
		Adjusted for age 11 IQ	0.23 (0.003)	0.05 (0.116)
		Left arcuate fasciculus FA	-0.19 (0.013)	-0.12 (0.006)*
		Splenium corpus callosum FA	0.16 (0.043)	0.01 (0.803)*
		Splenium corpus callosum MD	-0.17 (0.026)	-0.02 (0.567)*

Note. Gender and age at time of assessment statistically controlled. All beta values are standardized. All associations are in the direction of *ADRB2* SNP G alleles. * n = ~650

*3.5.2. Exploratory analyses: v*ascular risk factors

Controlling for age and gender, linear regression analyses showed an association between rs1042713 and self-reported diagnosis of high blood pressure (β = 0.09, *P* = 0.019), and a trend toward an association with rs1042714 (β = 0.07, *P* = 0.067). Neither SNP was associated with self-reported diabetes, stroke, high cholesterol or any other cardiovascular disease (all *P* > 0.05). The following analyses therefore controlled for high blood pressure in addition to age, gender and (where applicable in cognitive analyses only) age 11 MHT score.

3.5.3. Exploratory analyses: ADRB2 and white matter tract integrity

Fifty-two separate tests of association between *ADRB2* SNPs and white matter integrity variables were conducted (see Table 3.2), of which four were nominally significant at the *P* < 0.05 level. For rs1042713, there were associations with lower FA of the left arcuate fasciculus (β = -0.10, *P* = 0.021) and right anterior thalamic radiation (β = -0.09, *P* = 0.025). For rs1042714 there were associations with lower left arcuate fasciculus FA (β = -0.11, *P* = 0.007) and higher MD (β = 0.09, *P* = 0.037). None of the associations survived FDR correction.

Table 3.2. Linear *ADRB2* SNP associations with white matter integrity parameters, fractional anisotropy (FA) and mean diffusivity (MD).

	rs1042713 (G allele)				rs1042714 (G allele)			
	Fractional anisotropy		Mean diffusivity		Fractional anisotropy		Mean diffusivity	
White Matter Tract	β (p)	N	β (p)	N	β (p)	N	β (p)	N
General factor (g_{MD} or g_{FA})	-0.08 (0.072)	531	0.02 (0.638)	531	-0.07 (0.086)	529	0.03 (0.554)	529
Genu of the corpus callosum	-0.06 (0.152)	570	0.01 (0.773)	570	-0.05 (0.284)	568	0.01 (0.774)	568
Splenium of the corpus callosum	0.01 (0.837)	586	0.01 (0.836)	586	0.01 (0.772)	584	-0.02 (0.582)	584
Left arcuate fasciculus	**-0.10 (0.021)**	**562**	0.06 (0.148)	562	**-0.11 (0.007)**	**560**	**0.09 (0.037)**	**560**
Right arcuate fasciculus	-0.06 (0.211)	512	-0.01 (0.913)	512	-0.08 (0.079)	509	0.04 (0.359)	509
Left uncinate fasciculus	-0.03 (0.528)	502	-0.05 (0.305)	502	-0.03 (0.531)	500	0.00 (0.938)	500
Right uncinate fasciculus	-0.05 (0.210)	555	0.06 (0.198)	555	-0.06 (0.146)	553	0.07 (0.124)	553
Left anterior thalamic radiation	-0.05 (0.285)	488	0.02 (0.641)	488	0.00 (0.929)	486	0.02 (0.603)	486
Right anterior thalamic radiation	**-0.09 (0.025)**	**567**	0.07 (0.102)	567	-0.06 (0.123)	564	0.01 (0.793)	564
Left rostral cingulum	-0.08 (0.073)	567	0.04 (0.357)	567	-0.07 (0.100)	565	0.02 (0.716)	565
Right rostral cingulum	-0.02 (0.589)	574	0.00 (0.983)	574	-0.04 (0.346)	572	-0.03 (0.495)	572
Left inferior longitudinal fasciculus	0.03 (0.540)	586	-0.06 (0.134)	586	-0.06 (0.150)	584	0.01 (0.867)	584
Right inferior longitudinal fasciculus	-0.07 (0.072)	587	0.02 (0.610)	587	-0.07 (0.072)	587	0.02 (0.610)	587

Note. Gender, diagnosis of high blood pressure and age at time of MRI statistically controlled. All beta values are standardized. Associations significant at $P < 0.05$ are printed in bold-face. All associations are in the direction of *ADRB2* SNP G alleles.

3.5.4. Exploratory analyses: ADRB2 and cognitive performance

In exploratory analyses, analyses examined a range of cognitive phenotypes at age 73, as well as MHT performance at ages 11 and 70 only, at first unadjusted and then adjusted for age 11 ability. The attempted replication of findings reported by Bochdanovits et al., and Penke et al. (above) controlled for age, gender and (where applicable) age 11 intelligence. Controlling for age, gender and additionally for high blood pressure, no significant associations were evident in the present analyses between *ADRB2* SNPs and g, g_{Speed} and g_{Memory}. Table 3.3 shows the associations between *ADRB2* SNPs and specific cognitive task scores, not adjusted for age 11 MHT scores. There was no evidence of association between *ADRB2* and specific memory or visuospatial tasks. The *ADRB2* SNP rs1042713 showed a nominally significant association with better performance on Simple RT, in the direction of the G allele ($\beta = -0.09$, $P = 0.010$). The rs1042714 polymorphism was also associated with better Simple RT ($\beta = -0.08$, $P = 0.035$) and Matrix Reasoning performance ($\beta = 0.07$, $P = 0.041$), in the direction of the G allele.

Additionally controlling for age 11 MHT scores in order to test for associations with lifetime cognitive change, Table 3.4 shows that the *ADRB2* SNP rs1042714 showed a nominally significant association with poorer processing speed (g_{Speed}; $\beta = -0.07$, $P = 0.045$). When tests of association were conducted with specific processing speed tasks, rs1042713 was associated with faster Simple RT ($\beta = -0.09$, $P = 0.025$) but worse Symbol Search performance ($\beta = -0.07$, $P = 0.044$). The rs1042714 polymorphism was associated with worse performance on Digit Symbol Coding ($\beta = -0.09$, $P = 0.010$) and Symbol Search ($\beta = -0.08$, $P = 0.022$) tests. Note that when age 11 MHT scores were controlled for, the nominally significant associations between rs1042714, Matrix Reasoning and Simple RT in Table 3.3 attenuated to non-significance (Table 3.4).

Table 3.3. Linear *ADRB2* SNP associations with cognitive abilities, not adjusted for age 11 cognitive ability.

Cognitive measure	rs1042713 (G allele)		rs1042714 (G allele)	
	β (p)	N	β (p)	N
Moray House Test: age 11[a]	0.07 (0.070)	717	0.06 (0.109)	712
Moray House Test: age 70	0.05 (0.188)	756	0.03 (0.345)	753
General factor: intelligence (g)	0.01 (0.787)	751	-0.01 (0.846)	748
Digit Span Backwards	0.02 (0.633)	765	0.02 (0.608)	763
Matrix Reasoning	0.06 (0.106)	764	**0.07 (0.041)**	**761**
Block Design	0.01 (0.850)	761	-0.03 (0.447)	758
Letter-Number Sequencing	0.02 (0.531)	763	0.00 (0.958)	760
General factor: processing speed (g_{Speed})	-0.01 (0.813)	716	-0.04 (0.243)	714
Digit Symbol Coding	-0.03 (0.337)	761	-0.07 (0.060)	758
Symbol Search	-0.03 (0.347)	759	-0.05 (0.147)	756
Simple Reaction time (seconds)	**-0.09 (0.010)**	**751**	**-0.08 (0.035)**	**748**
Four Choice Reaction Time (seconds)	0.04 (0.280)	759	0.05 (0.156)	756
Inspection Time Total	-0.01 (0.877)	737	-0.01 (0.772)	735
General factor: memory (g_{Memory})	0.01 (0.741)	735	-0.01 (0.879)	733
Logical Memory	0.03 (0.442)	758	-0.00 (0.917)	756
Verbal Paired Associates	-0.01 (0.818)	741	-0.02 (0.643)	738
Spatial Span	-0.10 (0.787)	762	0.02 (0.622)	759

Note. Age at time of testing, gender and high blood pressure statistically controlled. All beta values are standardised. Associations significant at $P < 0.05$ are printed in bold-face. [a]Controlling for age at time of testing and gender only. All associations are in the direction of *ADRB2* SNP G alleles.

Table 3.4. Linear *ADRB2* SNP associations with cognitive abilities, adjusted for age 11 cognitive ability.

Cognitive measure	rs1042713 (G allele)		rs1042714 (G allele)	
	β (p)	N	β (p)	N
Moray House Test: age 70	0.00 (0.873)	710	0.00 (0.957)	705
General factor: intelligence (g)	-0.04 (0.159)	705	-0.05 (0.097)	700
Digit Span Backwards	-0.03 (0.455)	717	-0.02 (0.580)	712
Matrix Reasoning	0.03 (0.419)	716	0.06 (0.101)	711
Block Design	-0.03 (0.321)	713	-0.06 (0.102)	708
Letter-Number Sequencing	-0.01 (0.783)	716	-0.03 (0.400)	711
General factor: processing speed (g_{Speed})	-0.04 (0.258)	671	**-0.07 (0.045)**	**667**
Digit Symbol Coding	-0.06 (0.087)	714	**-0.09 (0.010)**	**709**
Symbol Search	**-0.07 (0.044)**	**712**	**-0.08 (0.022)**	**707**
Simple Reaction time (seconds)	**-0.09 (0.025)**	**704**	-0.06 (0.092)	699
Four Choice Reaction Time (seconds)	0.05 (0.152)	713	0.07 (0.054)	708
Inspection Time Total	-0.02 (0.559)	690	-0.03 (0.427)	686
General factor: memory (g_{Memory})	-0.04 (0.202)	689	-0.05 (0.111)	685
Logical Memory	-0.02 (0.612)	711	-0.05 (0.173)	707
Verbal Paired Associates	-0.04 (0.293)	695	-0.04 (0.318)	690
Spatial Span	-0.03 (0.449)	715	-0.01 (0.866)	710

Note. Age at time of testing, gender, high blood pressure and age 11 Moray House Test score statistically controlled. All beta values are standardised. Associations significant at $P < 0.05$ are printed in bold-face. All associations are in the direction of *ADRB2* SNP G alleles.

Linear regression models were used to explore associations between white matter tracts and cognitive measures that both showed nominally significant associations with *ADRB2* variants. As shown in Table 3.5, Simple RT was associated with FA of the right anterior thalamic radiation (β = -0.10, P = 0.024). The rs1042713 G allele was associated with both of these variables. Of the measures associated with the rs1042714 G allele, FA of the left arcuate fasciculus was associated with Digit Symbol Coding (β = 0.12, P = 0.001) and g_{Speed} (β = 0.012, P = 0.004). With FDR correction for all reported tests, the association between left arcuate fasciculus FA and Digit Symbol Coding remained statistically significant (FDR-adjusted P = 0.048). None of the other associations between white matter integrity and cognitive function survived FDR correction.

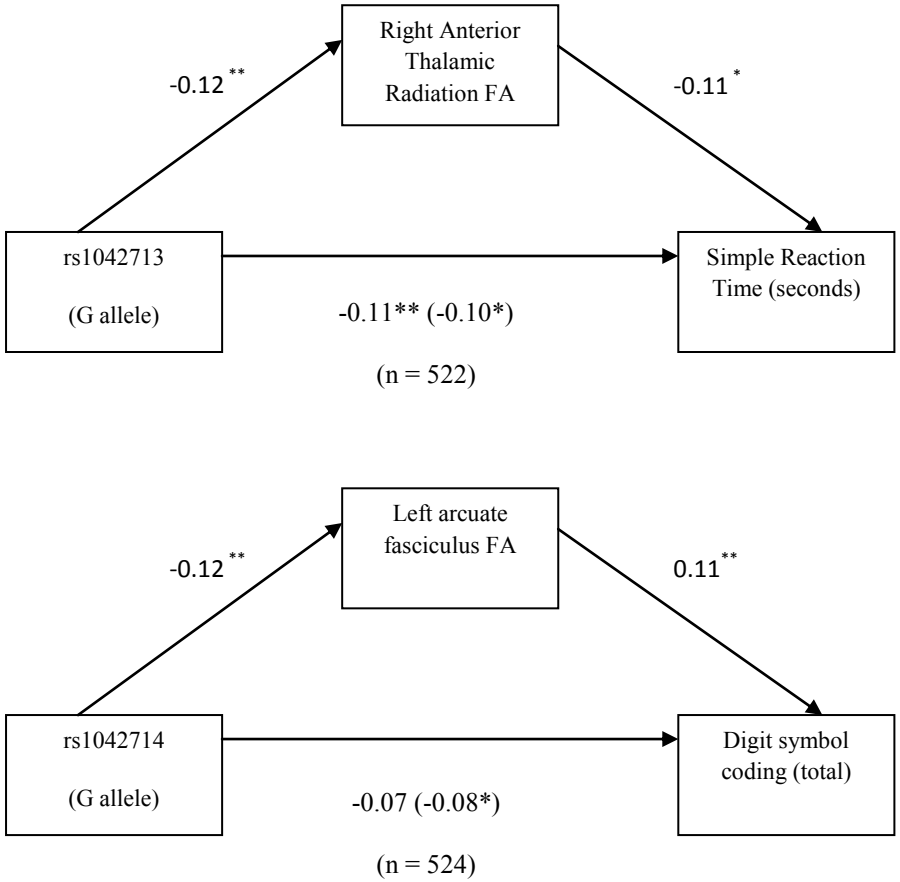
Neuroimaging and cognitive variables that both showed nominally significant associations with *ADRB2* SNPs were examined further (see Figure 3.2). Note that all genotype-phenotype associations attenuated to non-significance when corrected for FDR and this mediation analysis is therefore only exploratory. Bootstrapping statistics indicated that the association between rs1042713 and Simple RT was significantly mediated by right anterior thalamic radiation FA (bootstrapping point estimate coefficient i.e. indirect effect: <0.001 [95% C.I.s: <0.001 to 0.002]), because the bootstrapping confidence interval span did not contain zero (Preacher and Hayes, 2008). Left arcuate fasciculus FA was found to significantly mediate the association between rs1042714 and Digit Symbol Coding (indirect effect = -0.207 [95% C.I's: -0.519 to -0.043]), but not g_{Speed}.

Table 3.5. Associations between age 73 cognitive and white matter measures that are each associated with *ADRB2*.

White Matter tract	Symbol Search		Digit Symbol Coding		Simple Reaction time		General processing speed factor (g_{Speed})		Matrix Reasoning[a]	
	β (p)	N	β (p)	N	β (p)	N	β (p)	N	β (p)	N
Right anterior thalamic radiation (FA)	0.08 (0.062)	549	**0.13 (0.001)**	**549**	**-0.10 (0.024)**	**541**	**0.12 (0.003)**	**524**	**0.09 (0.038)**	**588**
Left arcuate fasciculus (FA)	0.05 (0.199)	546	**0.12 (0.001)**	**547**	-0.05 (0.273)	540	**0.12 (0.004)**	**522**	-0.00 (0.985)	584

Note. Age at time of testing, gender, high blood pressure and age 11 Moray House Test score statistically controlled. All beta values are standardised. Associations significant at $P < 0.05$ are printed in bold-face. [a]Not adjusted for age 11 cognitive ability.

Figure 3.2. Mediation analyses of the role of white matter integrity in the association between *ADRB2* SNPs and cognition, controlling for age, gender, diagnosis of high blood pressure and age 11 cognitive ability.



Note. Associations shown represent linear regressions between each variable. Note this mediation is in the context of attenuation to non-significance when corrected for multiple comparisons (FDR). All beta values are standardised. FA = fractional anisotropy. Values in brackets are betas before controlling for white matter integrity. All associations are in the direction of *ADRB2* SNP G alleles. *$P < 0.05$ **$P < 0.01$.

3.6. Discussion

3.6.1. Overview

In an earlier report employing the full LBC1936 sample and using Wave 1 data, (Bochdanovits et al., 2009), *ADRB2* SNPs were associated with cognitive tests performed at mean age 70 years. Specifically, rs1047213 and rs1042714 were associated with performance on two cognitive tests, namely Matrix Reasoning and the MHT. In a subsample study of the LBC1936 tested at mean age 73 years (Penke et al., 2010b), associations were found for rs1042714 with cognitive ability and particularly cognitive change from childhood to older age that were mediated by white matter integrity in the splenium of the corpus callosum. In the present study of only right-handed participants who completed cognitive testing, the majority of whom also underwent diffusion MRI, and with identical covariates, these associations with cognitive function and white matter integrity did not replicate.

3.6.2. Interpretation: failure to replicate previous reports

The effect sizes reported by Penke et al. were not found here and Bochdanovits et al. reported small coefficients, suggesting that associations may be sensitive to changes in sample size; specifically selection effects, restriction of range and random fluctuation. Compared with the associations reported by Bochdanovits et al., the effect sizes reported here are similar but slightly smaller, and with a smaller sample size did not attain statistical significance. The current study differed from Bochdanovits et al. in two ways: The earlier report assessed participants that attended Wave 1 and included left, right and ambidextrous-handed participants, whereas the current study included only right-handed individuals that attended Wave 2. One previous association that was reported by Bochdanovits et al. attenuated when analysis examined only right-handed participants, suggesting that this is a partial source of

discrepancy between the two reports. There is also evidence that another source of discrepancy may be selective attrition of more cognitively impaired individuals between Waves 1 and 2. It is important to note that Bochdanovits et al. reported opposing associations for the rs1042713 G allele; in the deleterious direction for younger participants, but in the protective direction for older participants. Their findings were therefore not unambiguous. Failure to replicate Penke et al. may reflect the limited number of participants in the original study compared to the current report. It is therefore possible that previous findings were type 1 errors.

3.6.3. Interpretation: exploratory analyses

In exploratory analysis of a range of age 73 white matter and cognitive phenotypes as well as MHT scores at ages 11 and 70, after controlling for gender, high blood pressure and age in days, rs1042713 was associated with faster Simple RT in the direction of the G allele. For rs1042714, associations were found with faster Simple RT and greater Matrix Reasoning performance in the direction of the G allele. After controlling for age 11 MHT scores, association between rs1042713 and faster Simple RT remained statistically significant in addition to an association with lower Symbol Search performance. When also adjusted for age 11 MHT scores, rs1042714 showed significant associations with lower Digit Symbol Coding, Symbol Search and g_{Speed} scores however previous associations with Simple RT and Matrix Reasoning attenuated to non-significance, indicating those effects were specific to cross-sectional ability at age 73 rather than cognitive ageing. In terms of white matter integrity, the G allele of rs1042713 was associated with lower FA of the left arcuate fasciculus and right anterior thalamic radiation tracts, while the G allele of rs1042714 was associated with lower FA/MD in the left arcuate fasciculus. When adjusted for multiple testing using FDR, all associations attenuated to non-significance.

Bearing in mind that correction for multiple testing could have been overly conservative, analyses explored, using bootstrapping, mediation of associations between *ADRB2* and cognitive task performances by specific white matter tracts where the uncorrected p-levels were indicative of such links. It should be emphasised that these analyses were cautiously exploratory in the context of genotype-phenotype associations that were non-significant when corrected for FDR. However, they could provide indications for future studies.

The relationship between rs1042714 and Digit Symbol Coding attenuated when left arcuate fasciculus FA was controlled for. This may suggest partial mediation (Salthouse 2010). Integrity of the arcuate fasciculus has been associated with performance on different cognitive domains (Schmithorst et al., 2005) and has been suggested to underlie parieto-frontal cortical integration, a proposed foundation of higher cognitive ability (Jung and Haier, 2007; Deary et al., 2010). The mediation reported here may therefore reflect the general role that this tract plays in subserving cognitive functioning in addition to the high sensitivity of the Digit Symbol Coding task in detecting even subtle cognitive dysfunction (Lezak et al., 2004). The association between rs1042713 and Simple RT showed evidence of being mediated by FA of the right anterior thalamic radiation tract. The strength of association between rs1042713 and Simple RT did not attenuate when relevant white matter integrity measures were controlled for however, and instead strengthened. This does not suggest true partial mediation; rather, these measures suppressed the relationship between rs1042713 and Simple RT (MacKinnon et al., 2000). It is counter-intuitive for the same allele to associate with faster Simple RT but lower integrity in associated white matter tracts. This suggests that this direct SNP-cognitive task association may instead be mediated by other related but distinct brain measures, for example integrity of the superior longitudinal fasciculus white matter tract which was not assessed here. It is also possible that this counter-intuitive finding reflects a spurious association, as reflected by its attenuation when corrected for FDR.

3.6.4. Limitations and future studies

A large number of association tests were conducted, and all significant associations attenuated once corrected for multiple testing using FDR. Whereas there are likely to be type 1 errors in the current study, given the number of associations conducted, it is also possible that correction for multiple testing is rejecting modest but true signals (Williams and Haines, 2011). Cognitive and water diffusion tensor phenotypes are individually highly correlated as indicated by the g, g_{Speed}, g_{Memory}, g_{MD}, and g_{FA} factors, which means that each association test does not represent an independent observation. This can make correction for multiple testing overly conservative (Nyholt, 2001). The current study therefore requires replication in independent cohorts.

3.6.5. Summary

The current study examined healthy older adults that were cognitively assessed at age 11 years and again in older age. *ADRB2* previously showed significant associations with cognitive ageing mediated by white matter tract integrity in a pilot study of the LBC1936 cohort. These associations were not replicated in this larger sample from the same cohort. Novel three-way associations were evident between *ADRB2* G alleles and specific cognitive and white matter tract integrity measures; however, all associations attenuated to non-significance upon correction for multiple testing. This correction could be considered conservative, so further research on functional *ADRB2* SNPs in large, independent samples is needed. This chapter provides proof of concept for subsequent Chapters 4-7 because analyses show significant mediating relationships between genetic, cognitive and brain imaging variables (based on unadjusted, nominal three-way associations).

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Chapter 4: Alzheimer's disease susceptibility genes *APOE* and *TOMM40*, and brain white matter integrity

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4.1. Abstract

An ε2/ε3/ε4 haplotype in the *APOE* gene has previously been significantly associated with cognitive, brain imaging, and Alzheimer's disease-related (e.g. age of onset) phenotypes. In the *TOMM40* gene, the rs10524523 ('523') variable length poly-T repeat polymorphism has more recently been associated with similar cognitive and AD-related phenotypes, although the allelic directions of these associations have varied between initial reports. Using diffusion MRI tractography, the current study aimed to investigate whether there are independent effects of *APOE* and *TOMM40* on human brain white matter integrity in a community-dwelling sample of older adults, the Lothian Birth Cohort 1936 (mean age = 72.70 years, standard deviation [SD] = 0.74, N approx. 640-650 for most analyses).

Some nominally significant effects were observed (i.e. covariate-adjusted differences between genotype groups at $P < 0.05$). For *APOE*, deleterious effects of ε4 'risk' allele presence (versus absence) were found in the right ventral cingulum and left inferior longitudinal fasciculus. To test for biologically independent effects of the *TOMM40* 523 repeat, participants were stratified into *APOE* genotype subgroups. In participants with the *APOE* ε3/ε4 genotype, effects of *TOMM40* 523 status were found in the left uncinate fasciculus, left rostral cingulum, left ventral cingulum, and a general factor of white matter integrity. In all four of these tractography measures, carriers of the *TOMM40* 523 'short' allele showed lower white matter integrity when compared with carriers of the 'long' and 'very-long' alleles. The majority of these effects survived correction for childhood intelligence test scores and cardiovascular disease history, though only the effect of *TOMM40* 523 on left ventral cingulum integrity survived correction for false discovery rate.

The effects of *APOE* in this older population are more specific and restricted compared with those reported in previous studies, and the effects of *TOMM40* on white matter integrity appear to be novel.

4.2. Introduction

APOE and TOMM40 genetic loci

This *APOE* ε and *TOMM40* 523 gene loci are detailed in Chapter 1 (*'Introduction'*) and Chapter 2 (*'Methodology'*).

4.2.1. Brain white matter integrity

Diffusion tensor MRI (DTI) and quantitative tractography were introduced in Chapter 3 ('*ADRB2*…') and are recapped here. Briefly, DTI allows examination of brain white matter microstructure *in vivo* in specific white matter tracts thought to relate to cognitive functions (Behrens et al., 2007; Pierpaoli et al., 1996). Fractional anisotropy (FA) is an example of a common DTI-derived metric, and reflects the level of directional coherence of water molecule diffusion (Pierpaoli et al., 1996). Specifically, FA measures are high in healthy, structurally intact, coherently organised white matter, but fall in diseased tissue.

There are significant deleterious changes in brain white matter integrity in people diagnosed with Alzheimer's disease (AD). Sexton et al. (2011) conducted a meta-analysis of 41 independent studies that had compared samples of people with clinical AD (typically based on DSM-4 or NINCDS-ADRDA diagnostic criteria; N = 617), with healthy controls (HC; N = 915), corrected for age and education (in years). They calculated an effect size, Hedge's g, reflecting the difference between groups divided by the pooled SD, and therefore corrected for any bias associated with small sample sizes. Sexton et al. reported significant, deleterious effects of AD group membership on 11 of 13 regions where brain white matter FA was assessed in different studies (Hedge's g range = -0.23 to -1.14; $P < 0.05$; 'Small' to 'Large' effect sizes).

White matter tract integrity shows significant non-pathological age-related change (e.g. Bartzokis et al., 2003, N = 252, mean age = 54.9 years, SD = 17.5, non-linear quadratic

coefficient for age on frontal lobe white matter integrity = 0.34, P <0.001), and may be associated with cognitive abilities by forming the basis for connecting brain networks, where lower integrity may hinder the efficient processing of information (Westlye et al., 2012a). Penke et al. (2010a), for example, reported that a general factor of white matter tract integrity FA (g_{FA}), constructed with principal components analysis (PCA), was significantly associated with a general factor of information processing speed (r = -0.24, P = 0.007) in a subsample of the LBC1936 dataset that had by that time undergone DTI (n = 132; aged around 73 years), adjusted for age and gender.

4.2.2. APOE, TOMM40 and brain white matter integrity

Associations between the *APOE* gene and white matter integrity have been investigated previously (see Gold et al., 2012 for a review of significant findings, and Table 4.1 which provides an outline). Observing Table 4.1, certain points are salient:

1. The majority of previous reports investigate samples with relatively wide age ranges. Heterogeneous age ranges in samples of older adults can be problematic; the correlation between age and white matter integrity is unlikely to be completely unique and rather may be via several processes *associated* with chronological age, such as amyloid-beta accumulation (Hofer and Sliwinski, 2001). Several studies statistically adjust for the effects of age. This only partially removes ageing effects; it is unlikely to control for the cumulative effects of all subtle age-related changes (Hofer and Sliwinski, 2001). This could result in spurious results because several brain imaging/cognitive phenotypes show change with age. Homogeneous age samples are therefore preferable.
2. Cardiovascular disease history is not consistently controlled for; significant associations between *APOE*/*TOMM40* genotypes and cardiovascular pathology could result in spurious or secondary association white matter integrity.

The largest previous study of *APOE* and brain white matter tract integrity had 203 participants (Westlye et al., 2012b; mean age = 47.6 years, SD = 14.9). That report found widespread differences in microstructural integrity depending on *APOE* status. Controlling for age and gender, ε3/ε4 carriers had lower white matter integrity (vs. ε3/ε3) in the brainstem, basal temporal lobe, internal capsule, anterior parts of the corpus callosum, forceps minor, superior longitudinal fasciculus, occipital and corticospinal motor pathways (Cohen's *d* range = 0.77 to 0.79; 'medium-large effects'). To date there appear no studies that have examined the independent effects of *TOMM40* 523 poly-T repeat genotype.

Table 4.1. Previous reports investigating the apolipoprotein-e *(APOE)* and diffusion tensor parameters.

Authors	*Imaging; measures (APOE analysis)*	*Sample (mean age; SD)*	*N*	*Covariates*	*Phenotypic association (direction of* ε4)	*Type 1 error adjustment*	*Notes & limitations*
Heise et al. 2011	Whole brain TBSS; FA & MD. (1. *APOE* ε4+ vs. -). (2. age*ε4 interaction).	Healthy young adults (28.6 years; ±4.20*) Healthy older adults (64.8; ±7.2*).	34 & 39	Age, sex, education, family history of AD & ACE-r scores (all n.s; p > 0.05 between genotypes).	FA ↓ & MD ↑; Cingulum, Corona Radiate, Corpus Callosum, External/Internal Capsules, Superior Longitudinal Fasciculus (overall sample).	Permutation testing (5000, $P < 0.05$).	No effect sizes reported. No age group*ε4 interaction ($P > 0.05$).
Honea et al. 2009	TBSS; FA (ε4+ vs. -).	Healthy older adults (73.4; ±6.3).	39	Age & gender (statistically controlled for); education & type-2 diabetes, frailty inventory (PPT) (n.s.).	FA ↓; (L) Parahippocampal gyrus *(coordinates -27X, -24Y, -23Z, voxel cluster size =N/A, P <0.001, n.s. after FWE correction).*	FWE rate (P <0.05).	Standard TBSS template may introduce error through discrepancy between template & subject ages (Chiang et al., 2012).
Persson et al. 2006	1. ROI; FA. Genu/splenium/body of corpus callosum 2. whole brain comparisons (ε4+ vs. ε3/ε3).	Healthy older adults (66.3; ±7.8*).	60	Age, sex, education, cardiovascular disorder history, blood pressure & associated medication & MMSE scores (n.s.)	i. FA ↓; splenium *(F [1, 56] = 7.39, M = .0.70 vs. 0.74], P = <0.01)* ii. FA ↓; anterior cingulum/occipito-frontal fasciculus *(cluster; 14X, 12Y, 28Z)*, splenium *(-4X, -44Y, 16Z),* & body of corpus callosum *(14X, 12Y, 28Z)*, & left hippocampus *(-26X, -38Y, -4Z) (further data N/A).*	None.	No whole-brain data reported & no correction for multiple testing.
Brown et al. 2011	Probabilistic tractography; level of inter-connectivity between specific brain structures and the rest of the brain, based on FA (age*ε4 interaction).	Healthy older adults (62.4; ±8.9).	55	Sex (controlled), education, family history of AD (n.s).	Age*ε4 deleterious interaction for FA; R precuneus *(ε4+ r = -0.64 vs. ε4- r = -0.13, P <0.001)*, L orbitofrontal cortex *(r = -0.64, vs. r = -0.13, P = 0.004)*, L supramarginal gyrus (*r = 0.54 vs. r = -0.35, P = 0.002),* R inferior temporal gyrus anteriorly *(r = -0.58 vs. -0.13, P <0.001).*	FDR (P <0.05).	*APOE* ε4 carriers had trajectories indicative of antagonistic pleiotropy; greater connectivity at younger ages, but sharper decline (vs. ε4-).
Bartzokis et al. 2006	ROI; T_2 relaxation rates. Orbitofrontal cortex, genu & splenium of corpus callosum (1. ε4+/ε2+ vs. ε3ε3) (2. age*ε4/ε2 interaction).	Healthy older adults (66.0; ±5.7*).	102	Age, education, sex, cardiovascular pathology, MMSE, white matter hyperintensities (n.s.).	1. ε2+ T_2 ↑: orbitofrontal cortex *(F [1, 101] = 5.39,* ε2+ *M = 15.97 vs. ε2- M = 15.65)*, genu *(F [1, 101] = 7.59, M = 15.87 vs. 16.24)* 2. ε2+*age interaction; less decline with age in orbitofrontal cortex *(ε2+ r = -0.011 vs. ε2-= -0.059).*	None.	No effects of ε4 allele presence.

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Chiang et al. 2012	1. ROI; FA, MD. Anterior/posterior corpus callosum (i.e. genu/splenium), cingulate, parahippocampus. 2. voxelwise whole-brain analysis (ε2+ vs. ε3/ε3).	Healthy older adults (68.5; ±10.9*).	39	Age (statistically controlled), gender, MMSE, T2 FLAIR white matter lesion load (all n.s.).	1. ε2+ FA ↑; posterior cingulate *(P <0.01, Cohen's D = 0.87; large effect size)*, anterior corpus callosum *(p 0.005, D = 0.95; large).* 2. ε2+ FA ↑; bilateral anterior thalamic radiations, corticopontine tracts, superior longitudinal fasciculus, right thalamus, cingulate and corpus callosum *(P <0.005, further data N/A)*.	None (*P* set to <0.005)	Large age range; 49 to 90 years. Note that TBSS is a more sensitive measure compared with defined ROI because it has less averaging of images, however is more sensitive to image/template misregistration (Chiang et al., 2012).
Ryan et al. 2010	ROI; FA & MD. Frontal lobe, parietal lobe, centrum semiovale, genu & splenium of corpus callosum, temporal stem. (1. ε4+ vs. ε4-) (2. age*ε4 interaction).	Healthy older adults (70.8; ±9.2*).	126	Gender, age, education (controlled), MMSE, white matter hyperintensities (n.s).	1. ε4+ FA ↓; frontal lobe (*F [1, 122] = 4.76, P <0.05, data N/A)*, splenium *(F [1, 122] = 4.76, P <0.05, data N/A).* 2. age*ε4, FA ↓; frontal lobe *(ε4+ r = -0.71 vs. ε4- r = -0.31),* temporal stem *(r = -0.59 vs -0.30),* genu *(r = -0.22 vs -0.22),* MD ↑; frontal lobe *(r = 0.73 vs 0.38)*, temporal stem *(r = 0.78 vs 0.42)*, parietal lobe (*r = 0.45 vs 0.55)*, centrum semiovale *(r = 0.52 vs. 0.58)* splenium *(r = 0.67 vs 0.48)*, genu *(r = 0.78 vs 0.42; all P < 0.05).*	None.	Stronger results for age*genotype interaction models compared with main effect models.
Bendlin et al. 2010	1. Voxel-wise comparison; FA & MD (*APOE* ε4+ vs. ε4 - & FH+ vs. FH-). 2. 'AD risk' model[a] (FH-/ε4-; FH-/ε4+; FH+/ε4-; FH+/ε4+).	Healthy older adults (56.77; ±5.35).	136	Age, sex, education (controlled).	1. No main effect of ε4+ vs ε4-. 2. Linearly lower FA with AD risk: L anterior & posterior corona radiate, uncinate, superior corona radiata, L tapetum, R external capsule, R posterior cingulum bundle, hippocampus and adjacent white matter *(all cluster sizes >20 voxels, P < 0.05).*	None.	No main effect of *APOE* independent of FH. Main effects of FH+ vs. FH- in most of the listed white matter regions (left). No effect sizes are reported.
Smith et al. 2010	Whole brain TBSS; FA (ε4+/FH+ vs. ε4-/FH-).	Healthy older females (61.0±1.2*).	65	Age, education (controlled).	FA significantly lower in inferior fronto-occipital/uncinate fasciculus, inferior longitudinal fasciculus, cingulum bundle (posterior & mid), cortico-pointinte tracts, splenium of the corpus callosum, subcallosal white matter *(no effect sizes reported; P < 0.01).*	None (*P* < 0.01).	

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Gold et al. 2010	Whole brain TBSS; FA, MD, DA and DR (ε4+/FH+ vs. ε4-/FH-).	Healthy older adults (58.8; ±5.8*).	57	Age, education (controlled).	FA ↓: fornix, inferior longitudinal fasciculus, inferior fronto-occipital fasiculus/uncinate fasciculus (main deleterious effect of ε4). MD ↑: genu of the corpus callosum, R inferior frontal /inferior longitudinal fasciculi. D_{ra} ↑: inferior longitudinal fasciculus, inferior frontal-occipital/uncinate fasciculi. DA ↓: rostral cingulum. No effect sizes reported.	None (*P* set at < 0.01)	Smith et al. & Gold et al. took subjects from the same sample (University of Kentucky imaging genetics project). It is unclear if subjects were included in both studies. The reports have different sample sizes. All association directions reflect lower integrity in the presence of ε4.
Felsky & Voineskos, 2013	Cingulum bundle FA. (ε4+ vs. ε4-).	Healthy older adults (mean age not detailed; range 18-86 years).	97	Age, gender, IQ (n.s.)	Significant deleterious age by ε4+ interaction *(F [4, 92] = 8.20, P = 0.005).*	None	
Ghaffar et al. 2011	Whole brain and then ROI (medial temporal lobes); FA & MD (1. *APOE* ε4+ vs. ε4-) (2. age*gender*disease duration*ε4 interactions).	People diagnosed with multiple sclerosis (43.9; ±10.0*).	90	Age and gender (controlled). Education, gender, disease duration, NART, expanded disability severity scores, white matter lesion volume (n.s.).	No significant main effects or interactions (all *P* values > 0.100).	None	Possible that the effects of *APOE* were subsumed by clinical pathology.

Note. TBSS = Tract Based Spatial Statistics, FA = fractional anisotropy, MD = mean diffusivity, D_{ra} = radial diffusivity, DA = axial diffusivity T_2 = transverse relaxation rate, AD = Alzheimer's disease, FWE = Family wise error, FDR = false discovery rate, n.s. = not statistically significant between groups at $P > 0.05$, L/R = left/right, N/A = not available/applicable, SD = standard deviation, ACE-r = Addenbrookes Cognitive Exam, PPT = physical performance test, M = mean, *r* = correlation coefficient, MMSE = mini mental state exam. FH = family history of AD, NART = National Adult Reading Test. All significant findings ($P < 0.05$) survived correction for multiple testing unless otherwise stated. All ages are in years. Higher FA indicates greater white matter integrity; higher MD, D_{ra} and T_2 indicate lower integrity. [a]Weighted contrasts tested for an additive effect of AD. * = weighted estimates.

4.2.3. The current study

The current study aims to investigate the effects of *APOE* and *TOMM40* on brain white matter integrity as assessed using quantitative tractography in a large, age-homogeneous sample of relatively healthy older people. Fourteen major projection, commissural and association fibre tracts were examined that have previously been significantly associated with cognitive abilities in this sample (Penke et al., 2012).

4.3. Methods

4.3.1. Sample and procedure

The LBC1936 recruitment, sample and procedure are detailed in Chapter 2 (‘*Methodology’*; Deary et al., 2007; 2012).

4.3.2. Childhood intelligence

The Moray House Test no.12 (MHT) completed at around age 11 years is described in Chapter 2 (‘*Methodology’*). MHT scores were adjusted for age in days at time of assessment, and standardised to an IQ score with a mean of 100 and a standard deviation of 15 for the whole LBC1936 sample.

4.3.3. Genotyping

The genotyping of the *APOE* ε2/ε3/ε4 and *TOMM40* 523 loci is detailed in Chapter 2 (‘*Methodology’*).

4.3.4. Diffusion MRI and tractography analysis

The core brain MRI procedure is described in Chapter 2 (‘*Methodology’*) and the DTI processing is broadly described in Chapter 3 (‘*ADRB2*…’). Both are detailed in the imaging protocol paper by Wardlaw et al. (2011). Principal components analysis (PCA) was performed on the tractography data to give a clear single factor model for FA (g_{fa}) that accounted for 38.8% of the overall variance, as described in Chapter 3 (‘*ADRB2*…’). General white matter integrity factors have previously been found to be associated with cognitive abilities in this sample (Penke et al., 2012; Penke et al., 2010a).

4.4. Statistical analysis

4.4.1. Statistical models

Three statistical models were used to investigate the effect of *APOE* and *TOMM40* genetic variants (the independent variables) on tract-averaged FA values (the dependent variables) for the fourteen tracts and g_{fa}. These models are detailed in Chapter 2 (‘*Methodology’*), however for ease are recapped in Table 4.2. Briefly, Model 1 adjusted for the covariates of gender and age in days at neuroimaging; significant effects were re-tested controlling for the additional covariate of age 11 IQ (‘Model 2’), and then effects that remained significant were re-tested controlling for self-reported cardiovascular disease histories (Table 4.2).

To protect against Type 1 errors, false discovery rate (FDR) was used to estimate the number of significant findings in the context of multiple testing (Benjamini et al., 1995). A Microsoft Excel program (Pike 2011) was used to conduct classical one-stage FDR based on associations with white matter integrity. All *P* values are raw unless stated as being FDR-adjusted. *P*-values < 0.05 were considered to be ‘nominally’ significant. Final results did not

differ whether FDR was applied separately to individual sets of analyses (i.e. A*POE* and then *TOMM40* sub-analyses), or to a collated list of all analyses.

Table 4.2. Final analytic strategies for *APOE* ε and *TOMM40* '523' poly-T repeat gene loci for Chapters 4-7.

	APOE ε	*TOMM40 '523'*
Gene locus:	rs7412 + rs429358 (simply 'ε genotype')	Poly-T repeat at rs10524523
Step 1:	ε4 present (vs. ε4 absent)	Overall effect of genotype? (S/S; S/L; VL/L; L/L; VL/VL) (in the whole sample)
Step 2:	ε3/ε4 (vs. ε3/ε3)	S/S; S/L*; L*/L* (in *APOE* ε3/ε4 genotype subgroup)
Step 3:	ε2 present (vs. ε3/ε3)	S/S; S/L*; L*/L* (in *APOE* ε3/ε3 genotype subgroup)
Covariate models:	Model 1: Age + Gender Model 2: Age + Gender + Age 11 IQ Model 3: Age + Gender + Age 11 IQ + Cardiovascular disease history	

Note. The term 'ε4 present' includes participants with ε3/ε4; ε2/ε4; ε4/ε4 genotypes pooled together, while 'ε4 absent' includes all other genotypes. 'ε2' present includes participants with ε2/ε3 and ε3/ε3 genotypes only. *TOMM40* 523 L* = L and VL alleles pooled.

4.4.2. APOE/TOMM40 analysis

The analytic strategies for the *APOE* ε and *TOMM40* 523 genetic loci are detailed in Table 4.2. Briefly, the first analytic step tested the effects of *APOE* ε4 allele presence vs. absence, i.e. pooled ε2/ε4, ε3/ε4 and ε4/ε4 genotypes versus pooled ε2/ε2, ε2/ε3 and ε3/ε3 (‘*Step 1’*). To test the effects of a single ε4 allele against a neutral genotype, ε3/ε4 was then compared with ε3/ε3 (‘*Step 2’*). The third analytic step tested genotypes which may be protective for neurodegenerative pathology, compared with the neutral genotype; i.e.; pooled ε2/ε3 and ε2/ε2 versus ε3/ε3 (*‘Step 3’*; Deary et al., 2004; Luciano et al., 2009a).

The variable-length poly-T repeat rs10524523 (‘523’) was split into three categories: ‘S’ (<20 T residues), ‘L (≥ 20) and ‘VL (≥ 30) (Lutz et al., 2010), of which the S allele may or may not be protective in terms of neurodegenerative pathology (Bruno et al., 2011a; Chu et al., 2011). In the first analytic step, in the whole sample, a GLM tested for a significant effect of *TOMM40* 523 genotype (i.e. S/S; S/L; L/L; L/VL; VL/VL; *‘Step 1’*). To investigate the effects of *TOMM40* 523 repeat length independent of biological variation in *APOE* genotype, analysis then focussed separately on two different *APOE* ε genotype subgroups; firstly participants with the ε3/ε4 genotype (*‘Step 2*’). Finally, analysis focussed on participants with the neutral *APOE* genotype (ε3/ε3) (*‘Step 3’*), because this eliminates variance associated with protective and risk *APOE* alleles (Roses et al., 2010).

In large samples of Caucasians, linkage between the *APOE* ε genotype and *TOMM40* 523 length (i.e. ε4 links primarily to ‘L’, ε3 primarily to ‘S’ or ‘VL’) is such that in the *APOE* ε3/ε3 genotype, relatively few L carriers would be predicted while in the ε3/ε4 genotype typically one L allele would be predicted in addition to either an S or VL allele (Linnertz et al., 2012). Slight errors in poly-T repeat length measurement may occur through PCR ‘slippage’ and this may result in repeat lengths that are close to the L/VL boundary being

incorrectly classified (Linnertz et al., 2012). To attempt to control for this, in Steps 2 and 3 the L and VL alleles were pooled into an 'L*' group; participants with the S/S genotype were compared with those carrying only one S allele (pooled S/L and S/VL; hereinafter S/L*), and also against participants carrying no S alleles (pooled L/L, L/VL and VL/VL; hereinafter L*/L*; Caselli et al., 2012). A GLM therefore tested for effects of S-allele group (S/S; S/L*; L*/L*) on brain white matter integrity variables in Steps 2 and 3 (*APOE* ε3/ε4 and ε3/ε3 subgroups respectively).

4.5. Results

4.5.1. Descriptive statistics

Of the 1091 total LBC1936 participants, 866 attended Waves 1 and 2. Of those 866, 731 underwent neuroimaging of which n = 700 completed the scans (reasons for non-completion generally included discomfort or claustrophobia). As described in Chapter 2 ('*Methodology'*), Individuals who reported being ambidextrous or left handed at either 'Wave 1' or 'Wave 2', reported history of dementia, had MMSE scores below 24 or did not complete the MMSE at Wave 2 were excluded. Overall, this left 675 participants, of which 642 and 652 participants had successful genotyping for *APOE* and *TOMM40*, respectively.

APOE had allele frequencies of ε2 = 7.4%, ε3 = 77.0% and ε4 = 15.6%, with genotype frequencies of: ε2/ε2 = 2 (0.3%), ε2 /ε3 = 77 (12.0%), ε2 /ε4 = 14 (2.2%), ε3/ε3 = 376 (58.6%), ε3/ε4 = 160 (24.9%), and ε4/ε4 = 13 (2.0%). *TOMM40* 523 had allele frequencies of S = 41.3%, L = 15.3% and VL = 43.4%, with genotype frequencies of S/S = 102 (15.6%), S/L = 94 (14.4%), S/VL = 240 (36.8%), L/L = 15 (2.3%), L/VL = 76 (11.7%) and VL/VL = 125 (19.2%). Exact tests confirmed that *APOE* and *TOMM40* were in Hardy-Weinberg equilibrium (*P*-values = 0.656 and 0.273, respectively).

4.5.2. APOE and quantitative tractography

For the *APOE* ε4 present vs. absent comparison, significant effects were found for two tracts, where presence of the ε4 allele was associated with poorer white matter integrity in the predicted direction (see Tables 4.3 and 4.5).

Step 1: The first significant effect was found in the right ventral cingulum (F [1, 570] = 5.48, $P = 0.020$, partial $\eta^2 = 0.010$). This survived correction for age-11 IQ (F [1, 535] = 4.17, $P = 0.042$, partial $\eta^2 = 0.008$), and cardiovascular disease history (F [1, 530] = 3.90, $P = 0.049$, partial $\eta^2 = 0.007$).

The second significant effect was found in the left inferior longitudinal fasciculus (F [1, 575] = 5.04, $P = 0.025$, partial $\eta^2 = 0.009$). This survived correction for age-11 IQ (F [1, 539] = 7.30, $P = 0.007$, partial $\eta^2 = 0.013$) and cardiovascular disease history (F [1, 534] = 7.00, $P = 0.008$, partial $\eta^2 = 0.013$).

Step 2: For the ε3/ε4 vs. ε3/ε3 comparison, two significant deleterious effects of ε3/ε4 were found (see Tables 4.3 & 4.5). This indicates that the presence of a single ε4 allele (compared with a neutral genotype) was associated with poorer white matter integrity, in the predicted direction.

Firstly, a deleterious significant effect of one ε4 allele was found in the left ventral cingulum (F [1, 471] = 4.33, $P = 0.038$, $\eta^2 = 0.009$), which survived correction for age 11 IQ (F [1, 441] = 4.06, $P = 0.044$, $\eta = 0.009$) but not cardiovascular disease history (F [1, 436] = 3.23, $P = 0.073$, $\eta^2 = 0.007$).

Secondly, the deleterious significant effect found in the left inferior longitudinal fasciculus (F [1, 481] = 4.15, $P = 0.042$, $\eta^2 = 0.009$) survived correction for age 11 IQ (F [1, 451] = 6.47, $P = 0.011$, $\eta^2 = 0.014$) and cardiovascular disease history (F [1, 446] = 6.75, $P = 0.010$, $\eta^2 = 0.015$).

Step 3: There was no main effect of the pooled ε2/ε3 and ε2/ε2 genotypes (vs. ε3/ε3) on the white matter integrity of any of the tracts analysed (see Table 4.3).

Table 4.3. Apolipoprotein-ε (*APOE*) and white matter integrity

White matter tract (FA)	*Step 1* ε4 allele presence (vs. absence) (d.f.) F statistics	*P*	Partial η^2	*Step 2* *APOE* ε3/ε4 (vs. ε3/ε3) (d.f.) F statistics	*P*	Partial η^2	*Step 2* *APOE* ε2/ε3 & ε2/ε2 (vs. ε3/ε3) (d.f.) F statistics	*P*	Partial η^2
General factor (g_{fa})	(1, 524) = 0.83	0.362	0.002	(1, 436) = 1.10	0.294	0.003	(1, 376) = 0.12	0.730	0.000
Genu of the corpus callosum	(1, 559) = 0.20	0.659	0.000	(1, 465) = 0.12	0.730	0.000	(1, 399) = 0.26	0.612	0.001
Splenium of the corpus callosum	(1, 575) = 0.07	0.788	0.000	(1, 480) = 0.34	0.336	0.001	(1, 409) = 0.00	0.985	0.000
Left arcuate fasciculus	(1, 552) = 0.00	0.987	0.000	(1, 461) = 0.16	0.689	0.000	(1, 393) = 0.62	0.432	0.002
Right arcuate fasciculus.	(1, 500) = 0.17	0.683	0.000	(1, 417) = 0.51	0.475	0.001	(1, 356) = 0.03	0.873	0.000
Left anterior thalamic radiation	(1, 479) = 0.00	0.962	0.000	(1, 400) = 0.10	0.754	0.000	(1, 341) = 0.00	0.957	0.000
Right anterior thalamic radiation	(1, 556) = 0.11	0.745	0.000	(1, 465) = 0.13	0.716	0.000	(1, 395) = 0.00	0.979	0.000
Left uncinate fasciculus.	(1, 492) = 1.57	0.212	0.003	(1, 408) = 1.56	0.212	0.004	(1, 348) = 0.00	0.956	0.000
Right uncinate fasciculus.	(1, 545) = 0.00	0.990	0.000	(1, 455) = 0.01	0.935	0.000	(1, 390) = 0.03	0.873	0.000
Left rostral cingulum	(1, 556) = 0.06	0.803	0.000	(1, 463) = 0.09	0.769	0.000	(1, 394) = 0.27	0.604	0.001
Right rostral cingulum	(1, 564) = 0.09	0.760	0.000	(1, 472) = 0.03	0.862	0.000	(1, 402) = 0.28	0.603	0.001
Left ventral cingulum	(1, 561) = 2.36	0.125	0.004	***(1, 471) = 4.33***	***0.038***	***0.009***	(1, 396) = 1.05	0.305	0.003
Right ventral cingulum	***(1, 570) = 5.48***	***0.020***	***0.010***	(1, 476) = 3.47	0.063	0.007	(1, 404) = 1.19	0.277	0.003
Left inferior longitudinal fasciculus	***(1, 575) = 5.04***	***0.025***	***0.009***	***(1, 481) = 4.15***	***0.042***	***0.009***	(1, 407) = 0.17	0.689	0.000
Right inferior longitudinal fasciculus	(1, 577) = 0.27	0.604	0.000	(1, 481) = 0.27	0.607	0.001	(1, 409) = 1.07	0.303	0.003

Note. Age in days at time of testing and gender statistically controlled. Associations significant at $P < 0.05$ are printed in bold-face and italics. FA = fractional anisotropy.

4.5.3. TOMM40 523 length and quantitative tractography

Step 1: In the whole sample, two significant effects of *TOMM40* 523 genotype were found (see Table 4.4). The first significant effect was found in the left ventral cingulum (F [5, 564] = 2.60, $P = 0.025$, partial $\eta^2 = 0.022$). This effect survived correction for additional covariates of age-11 IQ (F [5, 531] = 2.57, $P = 0.026$, partial $\eta^2 = 0.024$) and cardiovascular disease history (F [5, 526] = 2.79, $P = 0.017$, partial $\eta^2 = 0.026$). Post-hoc tests showed that the S/L

genotype had significantly lower FA compared with S/S (P = 0.029), S/VL (P = 0.008), L/L *(*P = 0.033) and L/VL (P = 0.003) genotypes (all other comparisons were $P > 0.05$).

The second significant effect was found in the right rostral cingulum (F [5, 568] = 2.99, P = 0.011, partial η^2 = 0.026). This effect survived correction for age-11 IQ (F [5, 533] = 3.73, P = 0.003, partial η^2 = 0.034) and cardiovascular disease history (F [5, 528] = 3.62, P = 0.003, partial η^2 = 0.033). Post-hoc tests showed that the S/L genotype had significantly lower FA compared with the S/VL (P = 0.011) and L/VL (P = 0.026) genotypes, with VL/VL having significantly lower FA compared with L/VL (P = 0.017; all other comparisons were $P > 0.05$).

To determine whether association between *TOMM40* 523 length and white matter tract integrity was driven by linkage with *APOE*, the above associations were re-tested controlling for presence of the ε4 allele. Controlling for age, gender and *APOE* ε4 presence, the main effect of *TOMM40* 523 length remained for both right cingulum FA (F [5, 547] = 3.00, P = 0.011, partial η^2 = 0.024) and left ventral cingulum FA (5, 544] = 3.45, P = 0.004, partial η^2 = 0.031).

Table 4.4. Translocase of outer mitochondrial membrane 40 (*TOMM40*) '523' length and white matter integrity.

White matter tract (FA)	Whole sample			*APOE* ε3/ε4 genotype			*APOE* ε3/ε3 genotype		
	(d.f.) F statistics	*P*	Partial η^2	(d.f.) F statistics	*P*	Partial η^2	(d.f.) F statistics	*P*	Partial η^2
General factor (g_{fa})	(5, 529) = 2.11	0.063	0.020	***(1, 122) = 4.95***	***0.028***	***0.039***	(2, 303) = 1.04	0.356	0.007
Genu of the corpus callosum	(5, 564) = 1.83	0.105	0.016	(1, 131) = 2.42	0.122	0.018	(2, 321) = 0.92	0.401	0.006
Splenium of the corpus callosum	(5, 580) = 0.20	0.963	0.002	(1, 136) = 1.56	0.214	0.011	(2, 331) = 0.21	0.810	0.001
Left arcuate fasciculus	(5, 557) = 0.64	0.669	0.006	(1, 131) = 0.92	0.339	0.007	(2, 317) = 0.62	0.539	0.004
Right arcuate fasciculus.	(5, 507) = 0.50	0.774	0.005	(1, 117) = 0.30	0.584	0.003	(2, 289) = 0.84	0.435	0.006
Left anterior thalamic radiation	(5, 481) = 1.68	0.139	0.017	(1, 110) = 2.68	0.104	0.024	(2, 277) = 0.19	0.826	0.001
Right anterior thalamic radiation	(5, 560) = 1.47	0.197	0.013	(1, 132) = 3.22	0.075	0.024	(2, 320) = 2.06	0.129	0.013
Left uncinate fasciculus.	(5, 594) = 1.61	0.157	0.016	***(1, 117) = 4.16***	***0.044***	***0.034***	(2, 279) = 0.49	0.616	0.003
Right uncinate fasciculus.	(5, 549) = 1.13	0.343	0.010	(1, 128) = 1.39	0.240	0.011	(2, 314) = 0.88	0.416	0.006
Left rostral cingulum	(5, 561) = 2.09	0.065	0.018	***(1, 133) = 5.51***	***0.020***	***0.040***	(2, 317) = 1.87	0.156	0.012
Right rostral cingulum	***(5, 568) = 2.99***	***0.011***	***0.026***	(1, 133) = 1.96	0.164	0.015	(2, 326) = 2.87	0.058	0.017
Left ventral cingulum	***(5, 564) = 2.56***	***0.025***	***0.022***	***(1, 136) = 13.35***	***<0.001***	***0.089***	(3, 322) = 0.72	0.487	0.004
Right ventral cingulum	(5, 576) = 0.75	0.584	0.006	(1, 137) = 0.00	0.980	0.000	(2, 326) = 0.83	0.439	0.005
Left inferior longitudinal fasciculus	(5, 580) = 0.88	0.492	0.008	(1, 138) = 0.12	0.725	0.001	(2, 330) = 0.83	0.436	0.995
Right inferior longitudinal fasciculus	(5, 581) = 0.66	0.655	0.006	(1, 138) = 0.37	0.547	0.003	(2, 331) = 0.81	0.448	0.005

Note. Age in days at time of testing and gender statistically controlled. Associations significant at $P < 0.05$ are printed in bold-face and italics. FA = fractional anisotropy.

Step 2: In ε3/ε4 carriers, four significant effects of *TOMM40* 523 length were found; these suggested a deleterious effect of the S/L* genotype versus L*/L* (see Tables 4.4 and 4.5). This indicates that possessing an S allele is associated with lower white matter integrity compared with possessing only L or VL alleles (as pooled into the 'L*' group). Note that the ε3/ε4 group had no S/S homozygotes.

The first significant effect was found for g_{fa} (F [1, 122] = 4.95, $P = 0.028$, partial $\eta^2 = 0.039$). This survived correction for additional covariates of age-11 IQ (F [1, 114] = 5.35, $P = 0.023$, partial $\eta^2 = 0.045$) and cardiovascular disease history (F [1, 109] = 5.84, $P = 0.017$, partial $\eta^2 = 0.051$).

The second significant effect was found in the left uncinate fasciculus (F [1, 117] = 4.16, P = 0.044, partial η^2 = 0.034), which did not survive the addition of the covariate of age-11 IQ (F [1, 108] = 3.68, P = 0.058, partial η^2 = 0.033). However, the initial significant effect remained when including cardiovascular disease history but not age-11 IQ as a covariate, (F [1,112] = 4.12, P = 0.045, partial η^2 = 0.035).

The third significant effect was found in the left rostral cingulum (F [1, 133] = 5.51, P = 0.020, partial η^2 = 0.040). This survived correction for additional covariates of age-11 IQ (F [1, 124] = 5.50, P = 0.021, partial η^2 = 0.042) and cardiovascular disease history (F [1, 119] = 5.83, P = 0.017, partial η^2 = 0.047).

The fourth and final significant effect was found in the left ventral cingulum (F [1, 136] = 13.35, P <0.001, partial η^2 = 0.089) and remained after correction for additional covariates of age-11 IQ (F [1, 125] = 14.40, P = <0.001, partial η^2 = 0.103) and cardiovascular disease history (F [1, 120] = 15.75, P = <0.001, partial η^2 = 0.116).

Step 3: No significant effects of the *TOMM40* 523 poly-T repeat length polymorphism were found at Step 3 (i.e. in the ε3/ε3 genotype; see Table 4.4).

4.5.4. Correction for multiple testing

With FDR correction, all nominally significant (uncorrected) effects attenuated to non-significance except for the main effect of *TOMM40* 523 S/L* vs. L*/L* on left ventral cingulum FA in *APOE* ε3/ε4 carriers (Models 1, 2 and 3; FDR-adjusted P-values all = 0.017).

Table 4.5. Apolipoprotein-e (*APOE*), translocase of outer membrane 40 *(TOMM40)* '523' loci and white matter integrity; nominally significant findings ($P < 0.05$ in Table 4.3, and Table 4.4 subgroup analyses) adjusted for additional covariates.

		Adjusted for age 11 IQ							Additionally adjusted for cardiovascular disease history						
					Contrasts							Contrasts			
	White matter tract (FA)	(d,f) F statistics	*P*	Partial η^2	n	Est. mean (95% C.I's)	n	Est. mean (95% C.I's)	(d,f) F statistics	*P*	Partial η^2	n	Est. mean (95% C.I's)	n	Est. mean (95% C.I's)
APOE						ε4+		ε4-					ε4+		ε4-
ε4 + vs. ε4 – comparison	Right ventral cingulum	(1, 535) = 4.17	0.042	0.008	156	0.285 (0.279; 0.192)	384	0.293 (0.289; 0.297)	(1, 530) = 3.90	0.049	0.007	156	0.285 (0.279; 0.292)	384	0.293 (0.289; 0.297)
	Left inferior longitudinal fasciculus	(1, 539) = 7.30	0.007	0.013	157	0.392 (0.385; 0.399)	387	0.404 (0.399; 0.408)	(1, 534) = 7.00	0.008	0.013	157	0.392 (0.385; 0.399)	387	0.404 (0.399; 0.408)
TOMM40						S/L*		L*/L*					S/L*		L*/L*
'523' poly-T repeat length genotype, in *APOE* ε3/ε4 subgroup	General factor (g_{fa})	(1, 114) = 5.35	0.023	0.045	67	-0.295 (-0.543; -0.047)	52	0.143 (-0.138; 0.425)	(1, 109) = 5.84	0.017	0.051	67	-0.309 (-0.559; -0.059)	52	-0.161 (-0.124; 0.446)
	Left uncinate fasciculus	(1, 108) = 3.68	0.058	0.033	65	0.325 (0.317; 0.333)	48	0.337 (0.328; 0.346)	(1, 112) = 4.12*	0.045*	0.035*	68	0.324 (0.317; 0.332)	53	0.337 (0.328; 0.345)
	Left rostral cingulum	(1, 124) = 5.50	0.021	0.042	71	0.428 (0.417; 0.439)	58	0.447 (0.435; 0.459)	(1, 119) = 5.83	0.017	0.047	71	0.428 (0.417; 0.439)	58	0.448 (0.436; 0.460)
	Left ventral cingulum	(1, 125) = 14.40	<0.001	0.103	71	0.280 (0.271; 0.289)	59	0.306 (0.296; 0.315)	(1, 120) = 15.75	<0.001	0.116	71	0.279 (0.270; 0.316)	59	0.307 (0.297; 0.316)

Note. Age in days at time of testing and gender statistically controlled. Est. mean = estimated marginal mean difference adjusted for applicable covariates. FA = fractional anisotropy *Age-11 IQ not included as a covariate. *TOMM40* 523 'S' = 'Short' allele, 'L*' = pooled 'Long' and 'Very-long' alleles.

4.6. Discussion

4.6.1. Overview

The current study investigated the effects of variants in two genes upon brain white matter tract integrity in a large sample of non-demented, community-dwelling people in their early 70s. These gene loci were the *APOE* ε haplotype (commonly and herein referred to as 'ε genotype') and the *TOMM40* 523 poly-T repeat. The current report is the largest examination of the *APOE* locus and white matter integrity in a single study (Gold et al., 2012; Westlye et al., 2012b) and therefore adds a significant amount of new data to the literature. No previous studies have examined *TOMM40* 523 in relation to this phenotype.

The current study found significant effects of the *APOE* ε4 risk allele in the predicted deleterious direction on: i) the right ventral cingulum; and ii) the left inferior longitudinal fasciculus. The ventral cingulum is a parieto-occipital tract connecting the cingulate cortex with parahippocampal gyri and terminating in the anterior part of the medial temporal lobes. The left inferior longitudinal fasciculus connects occipital and temporal areas including the hippocampus (Catani and Thiebat de Schotten, 2008).

TOMM40 523 poly-T repeat length genotype had significant effects in the whole sample and in the subgroup of participants that possessed the *APOE* ε3/ε4 genotype. In the whole sample, significant effects were found in the left ventral cingulum and the right rostral cingulum, both primarily driven by the S/L genotype being associated with significantly lower FA compared with other *TOMM40* 523 length genotypes. These significant associations survived statistical correction for presence of the *APOE* ε4 allele, however were not significant in the subgroup of participants with the 'neutral' ε3/ε3 genotype, suggesting they are either i) unlikely to be truly independent of *APOE* genotype, and reflect type 1 error,

or ii) sensitive to reductions in sample size. Larger samples of individuals with the ε3/ε3 genotype would be required to address this further.

In *APOE* ε3/ε4 carriers, a significant deleterious effect of possessing an S allele (versus not) was found in: the i) left uncinate fasciculus; ii) left rostral cingulum; iii) left ventral cingulum; and iv) general factor of white matter integrity (g_{fa}). The uncinate fasciculus connects the anterior temporal lobe to medial and lateral orbitofrontal cortex, whereas the rostral cingulum projects from the cingulate to orbitofrontal cortices (Catani and Thiebat de Schotten, 2008). The g_{fa} parameter, which is determined from PCA of the tract-averaged FA values for twelve major fibre pathways in each subject, shows that the integrity of white matter is to a substantial degree shared across tracts throughout the brain, possibly indicating shared influences (Lopez et al., 2012; Penke et al., 2012).

The majority of reported associations remained when corrected for history of cardiovascular disease (e.g. history of stroke, etc.) and childhood intelligence. Corrected for multiple testing, only the deleterious effect of the *TOMM40* 523 S allele (vs. pooled non-carriers, in the *APOE* ε3/ε4 subgroup analysis) upon left ventral cingulum integrity remained significant. White matter diffusion tensor phenotypes are highly correlated as indicated by the g_{fa} factor. This can make correction for multiple testing overly conservative; therefore, for different reasons, nominal and adjusted effects require caution in their interpretation (Nyholt et al., 2011). All findings reported therefore require replication in large independent samples.

4.6.2. Interpretation: APOE ε genotype

It is unclear why the left inferior longitudinal fasciculus and right ventral cingulum would be particularly vulnerable to the effects of the *APOE* ε4 allele, and not the integrity of other white matter tracts. Having noted this, it is important to consider how these discreet and restricted effects might be explained mechanistically. White matter changes occur in the

healthy ageing brain but usually with an anterior-posterior gradient, in contrast to AD pathology which affects more posterior, temporal regions first (Bendlin et al., 2010; Bennett et al., 2010). The hippocampus for example shows the earliest evidence of amyloid-β and neurofibrillary tangle accumulations (Braak and Braak, 1991). The current study partially replicates previous associations between ε4 and tracts associated with temporal lobe structures before correction for multiple testing; specifically the inferior longitudinal fasciculus (Gold et al., 2010; Smith et al., 2010), and ventral cingulum (Smith et al., 2010; 'posterior cingulum'). It is possible that the effects of *APOE* seen here are indicative of prodromal AD in the current sample. However, this does not account for why no effects were found in other tracts associated with temporal lobe structures.

The current study is the largest assessment of *APOE* and white matter integrity to date and reports more circumscribed effects of ε4 allele possession compared with previous reports (Gold et al., 2012). This could relate to the large sample size and relatively homogenous age ranges assessed here compared with previous studies. Larger samples are likely to be more reliable, and wide age ranges across genotypes could include the cumulative influence of subtle age-related processes that affect the phenotype of interest; statistically controlling for age may not completely eradicate these effects (Hofer and Sliwinski, 2001). Discrepancies may also relate to differences between image analysis methods (e.g. TBSS; Westlye et al., 2012b) vs. probabilistic neighbourhood tractography used here). The current study does not interrogate white matter integrity across the whole brain and therefore cannot exclude the possibility of significant ε4 effects in regions that were not assessed, such as the fornix. Regardless, the nominally significant effects observed here were weaker and more specific than would have been expected when compared with the 'medium-large' effects reported previously (Westlye et al., 2012b).

4.6.3. Interpretation: TOMM40 523 poly-T repeat

Associations between the *TOMM40* 523 poly-T repeat and white matter integrity have not been investigated by previous studies. The above results indicate an independent deleterious effect of the *TOMM40* 523 S allele in *APOE* ε3/ε4 but not ε3/ε3 genotypes. This association is independent in the sense that any deleterious effects of *TOMM40* 523 S allele possession (vs. non-possession) cannot be causally attributed to linkage with *APOE* ε alleles, because a specific stable subgroup was analysed. Similar to *APOE*, the tracts that showed negative association with the S allele have been implicated in early AD pathology; the (left) uncinate fasciculus, (left ventral) cingulum and (left rostral) cingulum (Gold et al., 2010; Heise et al., 2011; Smith et al., 2010). As with *APOE*, it is unclear why these specific tracts (and not others) would be exclusively affected by *TOMM40* 523 length.

A significant effect was found for g_{fa}. This suggests that the *TOMM40* 523 repeat may influence processes that are sufficiently pleiotropic to affect general white matter integrity. Lopez et al. (2012) conducted a pathway analysis on g_{fa} based on the current sample dataset (n = 535). The authors used the web-based Gene Set Enrichment Analysis Toolkit (WebGestalt; Duncan et al., 2010; Zhang et al., 2005) to conduct enrichment analysis for gene ontology on genes that were significantly associated with g_{fa} (n = 173 genes, $P < 0.01$). Twenty-three ontology categories had enriched gene numbers, the most significant of which were "calcium-dependent cell-cell adhesion" ($P = 1.15 \times 10^{-11}$) and "synapse assembly" ($P = 5.20 \times 10^{-8}$). Speculatively, the nominally significant association between *TOMM40* 523 repeat length and g_{fa} may relate to these ontologies. Lopez et al. found that *TOMM40* did not reach gene significance based on 19 SNPs; note, however, that report did not directly assess variation in the poly-T repeat locus.

Significant effects of *TOMM40* 523 length were found only in the *APOE* ε3/ε4 genotype. The S allele may modulate the toxic effects of *APOE* ε4 allele presence (Bruno et

al., 2011a). In the current study this appears to be the case, with a negative effect of the S allele. This is consistent with the deleterious effect of the S allele on AD diagnosis risk in ε3/ε3 carriers as reported by Cruchaga et al. (2011), but inconsistent with studies that report protective effects on age of AD onset in ε3/ε4 carriers (Roses et al., 2010), specific normalized grey matter volumes in ε3/ε3 carriers (Johnson et al., 2011), or null effects on cognitive ageing in ε3/ε4 and ε4+/- groups (Schiepers et al., 2012). Crenshaw et al. (2013) report a protective effect of the S allele on the age of onset of mild cognitive impairment in ε3/ε4 participants, but in contrast a deleterious effect of the S/S genotype > S/VL genotype > VL/VL genotype in the ε3/ε3 and ε2/ε3 genotype. It is unclear what drives these differing directions, but the effect was significant on age of onset distributions in 106 conversion events using standard neuropsychological tests in a series of 508 Caucasians ascertained prospectively.

4.6.4. Limitations and future studies

This study conducted a large number of association tests which assessed significantly inter-correlated brain imaging phenotypes (as reflected by the g_{FA}, for example); this may make correction for multiple testing overly conservative, as discussed in Chapter 3 ('*ADRB2*…').

Better understanding of the functional significance of *TOMM40* 523 repeat length and its relationship with *APOE* may inform age-related phenotypic data. Bekris et al. (2012) conducted functional analysis of different poly-T repeat haplotypes on reporter assay expression levels in SHSY5Y, HepG2 and U118 neuronal cell lines. Results indicated that the *TOMM40* 523 poly-T repeat had measurable effects on enhancer/silencer activity, and that the direction of its effect on expression depends on the cell type and specific haplotype content. Further study of poly-T repeat function and significance is required in large samples,

including those with AD, *APOE* data, and age of onset data (Bekris et al., 2011; Hedskog et al., 2012; Cruchaga et al., 2011).

4.6.5. Summary

The current study found relatively circumscribed nominal significant effects of the *APOE* ε and *TOMM40* 523 gene loci on brain white matter integrity in the LBC1936. The majority of these associations attenuated when corrected for multiple testing. Replication in large independent samples is therefore required.

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Chapter 5: Alzheimer's disease susceptibility genes *APOE* and *TOMM40*, and hippocampal volume

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5.1. Abstract

The *APOE* ε and *TOMM40* rs10524523 ('523') variable length poly-T repeat gene loci have been significantly and independently associated with Alzheimer's disease (AD) related phenotypes such as age of clinical onset. Hippocampal atrophy has been significantly associated with memory impairment, a previously reported characteristic of AD. The current study aimed to test for independent effects of *APOE* ε and *TOMM40* '523' genotypes on hippocampal volumes as assessed by brain structural MRI in a relatively large sample of community-dwelling older adults. As part of a longitudinal study of cognitive ageing, participants in the Lothian Birth Cohort 1936 underwent genotyping for *APOE* ε2/ε3/ε4 status and *TOMM40* '523' poly-T repeat length, and detailed structural brain MRI at a mean age of 72.70 years (Standard deviation [SD] = 0.74, N range = 624 to 636). No significant effects of *APOE* ε or *TOMM40* 523 genotype were found on hippocampal volumes when analysed raw, or when adjusted for either intracranial or total brain tissue volumes. In summary, in a large community-dwelling sample of older adults, analyses found no effects of *APOE* ε or *TOMM40* 523 genotypes on hippocampal volumes. This is discrepant with some previous reports of significant association between *APOE* and left/right hippocampal volumes, and instead echoes other reports that found no association. Previous significant findings may partly reflect type 1 error. Future studies should carefully consider: 1) their specific techniques in adjusting for brain size; 2) assessing more detailed sub-divisions of the hippocampal formation; and 3) testing whether significant *APOE*-hippocampal associations are independent of generalised brain atrophy.

5.2. Introduction

Dementia is a growing world-wide problem, and it is important to understand the risk factors and mechanisms underlying the disease. The major and most common sub-type of dementia is Alzheimer's disease (AD; Rocca et al., 2011). A key brain region involved in the illness is the hippocampus, and hippocampal volumetric atrophy has been used as an indicator of AD risk (Potkin et al., 2009; Lind et al., 2006). Two genetic risk factors are in the *APOE* and *TOMM40* genes (Roses et al., 2010). The present study concerns the association between specific variants in these genes and hippocampal volume in non-demented older people, in an attempt to investigate further possible links between these candidate genes and a prominent brain imaging phenotype thought to be indicative of AD risk. Having detailed the *APOE* ε and *TOMM40* '523' poly-T repeat gene loci in Chapter 1 ('*Introduction'*) and Chapter 2 ('*Methodology'*), the following sections detail the hippocampus and its role in cognitive ability/AD.

5.2.1. The hippocampus, Alzheimer's disease and cognitive ability

The hippocampal formation of the brain includes the dentate gyrus, subiculum, entorhinal cortex, cornu ammonis (CA) areas 1-4 and the hippocampus proper (Braak and Braak, 1991; Amaral and Lavenex, 2006**).** The hippocampus itself occupies the floor of the temporal horn of the lateral ventricle. It is typically 4.0 to 4.5 cm long and around 1.5 cm wide (Duvernoy, 2006).

Braak and Braak (1995) outline stages of neurofibrillary change in AD pathophysiology, based on the observed accumulations of neurofibrillary tangles, neuropil threads and amyloid beta plaques. Neurofibrillary changes are associated with neuronal loss in the hippocampal formation because accumulations aggregate and impede inter/intra-neuronal function (Geodert, 1996), and forms the basis for neuropathological diagnosis of

AD (Hyman, 1997). AD-like neurofibrillary changes have been reported in the human brain in the absence of significant cognitive decline (Ohm et al., 1995; Simic et al., 1997), and are apparent first in the hippocampal formation (Braak and Braak, 1991; Price and Morris, 1999).

Hippocampal atrophy is associated with a common symptom of AD, namely memory impairment (Potkin et al., 2009; Lind et al., 2006). For example, Laakso et al. (1995) reported that in 32 participants with probable AD (mean age = 69.0, SD = 8.0), left hippocampal volume correlated significantly with immediate (r = 0.39, P = 0.029) and 30-minute delayed verbal memory scores (r = 0.50, P = 0.003). Similar findings have been reported in independent samples of individuals with very mild, or clinically diagnosed AD (e.g. Stoub et al., 2010), and in neuropsychological samples of individuals with relatively localised hippocampal lesions (e.g. Bechara et al., 2002).

5.2.2. APOE ε and hippocampal volume

Cross-sectional hippocampal volumes and *APOE* ε genotype in healthy older adults has been examined by a number of small (N range = 20 to 134) brain MRI studies, which vary between showing significant and null effects (e.g. Plassman et al., 1997; Richter-Schmidinger et al., 2011; Jak et al., 2007; Tupler et al., 2007; Chiang et al., 2011) and a few much larger reports including several hundred participants, which also show varying results (i.e. N > 150; Lemaitre et al., 2005; Den Heijer et al., 2002; Cherbuin et al., 2008; Panizzon et al., 2010; Ferencz et al., 2013; Hostage et al., 2013). These larger reports are likely to be more reliable, and are summarised in Table 5.1. Specifically, possession of the *APOE* ε4 allele has occasionally been significantly associated with lower hippocampal volumes in cross-sectional samples of older adults.

Table 5.1. Summary of previous large cross-sectional studies (i.e. N > 135) examining *APOE* ε4 genotype and hippocampal volumes in non-demented, community dwelling older adults.

Authors	Technique used in correcting hippocampal volumes for head size	Sample N	Mean age in years (Standard Deviation)	Covariates	Main findings	Statistics (hippocampal volumes)
LeMaitre et al., 2005	Hippocampal volumes expressed as a percentage fraction of intracranial volume.	750	69.4 (2.9)	Gender (statistically controlled), age, education, diagnosis of hypertension, Mini Mental State Exam score (no group differences; $P > 0.05$)	Significant deleterious effect of ε4/ε4 genotype (vs. non-ε4 carriers).	Left: ε4/ε4 = 0.23%, vs. non-ε4/ = 0.26% ($P < 0.001$). Right ε4/ε4 = 0.22% vs. non-ε4 = 0.24% ($P = 0.006$).
Den Heijer et al., 2002	Midsagittal area included as model covariate.	949	72.3 (7.0)	Age, gender (controlled).	Significant deleterious effect of ε4 allele presence (vs. non-ε4 carriers).	Left: ε4+ difference to ε3/ε3 genotype = -0.11 millilitres. Right: ε4+ difference to ε3/ε3 genotype = -0.11 milliliters.
Cherbuin et al., 2008	Intracranial volume included as model covariate.	331	62.6 (1.4)	Age, gender, education (controlled).	No significant main effects of ε4 allele (P > 0.05).	-
Panizzon et al., 2010	Intracranial volume included as a model covariate.	375	55.9 (2.6)	Relatedness between twins, handedness, age (controlled).	No significant main effects of ε4 allele (P > 0.05).	-
Ferencz et. al., 2012	Intracranial volume included as a model covariate.	424	69.9 (8.6)	Age (controlled).	No significant main effects of ε4 allele (P > 0.05).	-
Hostage et al., 2012	Intracranial volume included as a model covariate.	198	76.0 (0.5)	Age (controlled).	No significant main effects of ε4 allele (P > 0.05).	-

Note. The age mean and standard deviation data provided above for Lemaitre et al., Den Heijer et al. and Hostage et al. are weighted estimates; see those reports for exact age data.

5.2.3. TOMM40 '523' and hippocampal volume

TOMM40 523 poly-T repeat length is linked to *APOE* genotype, and it would appear that only one study has so far examined the independent effects of the *TOMM40* 523 gene locus on hippocampal volumes in healthy older adults. Johnson et al. (2011) tested for an effect of poly-T repeat length genotype on a whole-brain voxel-wise comparison of grey matter volumes in participants with the *APOE* ε3/ε3 genotype (N = 117, mean age = 55.42 years, SD = 6.00), and found no significant effect of poly-T repeat genotype on hippocampal volume. A recent large-scale genome wide association study in healthy adults (N = 5776) reported no significant associations with SNPs in the *TOMM40* gene however did not directly analyse the *TOMM40* 523 poly-T repeat locus (Stein et al., 2012).

5.2.4. The current study

The Lothian Birth Cohort 1936 (LBC1936; Deary et al., 2007) is large group of relatively healthy older adults of a narrow age range, that have undergone detailed brain MRI and *APOE*/*TOMM40* genotyping. The current study aims to add to the literature by investigating: 1) the effects of *APOE* ε in a large age-homogeneous sample of generally healthy older adults, and 2) the independent effects of the *TOMM40 523* poly-T repeat length, on hippocampal volumes in the LBC1936.

5.3. Methods

5.3.1. Sample and procedure

The LBC1936 recruitment, sample and procedure are detailed in Chapter 2 ('*Methodology'*; Deary et al., 2007; 2012).

5.3.2. Childhood intelligence

The Moray House Test no.12 (MHT) completed at around age 11 years is described in Chapter 2 (‘*Methodology’*). MHT scores were adjusted for age in days at time of assessment, and standardised to an IQ score with a mean of 100 and a standard deviation of 15 for the whole LBC1936 sample.

5.3.3. Genotyping

The genotyping of the *APOE* ε2/ε3/ε4 and *TOMM40* 523 loci is detailed in Chapter 2 (‘*Methodology’*).

5.3.4. Brain MRI analysis

The core brain MRI procedure is described in Chapter 2 (‘*Methodology’*; Wardlaw et al., 2011). This protocol paper states that left and right hippocampal volumes were semi-automatically segmented using FSL’s FIRST automated segmentation program (http://fsl.fmrib.ox.ac.uk) with manual correction, registered using FSL FLIRT to a locally developed template of similarly older brains (i.e. aged around 70 years). Final results were visually assessed for accuracy and manually edited using Analyse 9.0 where appropriate (www.analyzedirect.com). Further details are provided in the protocol paper by Wardlaw et al. (2011).

This method of semi-automated hippocampal segmentation has been validated against the ‘gold-standard’ approach of manual tracing – which, while very accurate (assuming a degree of technical expertise), is extremely time consuming (Wardlaw et al., 2011). Specifically, the automatic FSL segmentation results were compared with 120 manually traced hippocampal segmentations, and automated results were not considered accurate

enough to be used without closer examination. Automated results were therefore visually inspected for each participant, and corrected manually where necessary.

Intracranial volume measurements were obtained semi-automatically using the T2*-weighted sequence. Total brain tissue volumes were obtained using T2*-weighted with FLAIR. The volume of cerebrospinal fluid, venous sinuses and meninges was used to estimate total brain tissue volume by subtracting it from intracranial volume (Wardlaw et al., 2011). Further details for these measurements are available from Wardlaw et al. (2011).

5.4. Statistical analysis

5.4.1. Covariate models & APOE/TOMM40 statistical analysis

The covariate models and analytic strategies for the *APOE* and *TOMM40* gene loci were described in Chapter 2 ('*Methodology'*), and briefly recapped in Chapter 4 ('*...White Matter Integrity*'). All initially-reported *P*-values are raw (and then FDR-adjustment is applied), and *P*-values < 0.05 are considered nominally significant.

5.4.2. Hippocampal volume; statistical analysis

It is important to correct (commonly and herein 'normalize') left and right hippocampal volumes relative to an individual's head or brain size. To this end, left/right hippocampal volumes were analysed in three separate ways, controlling for age and gender throughout. First, left/right hippocampal volumes were examined raw. Second, hippocampal volumes were analysed with intracranial volume as an additional model covariate, reflecting maximum lifetime brain volume as a general proxy for head size. Finally, hippocampal volumes were analysed with current total brain tissue volume as an additional model covariate, to ensure

that any significant genetic associations with hippocampal volume were not reflective of more general brain atrophy (Free et al., 1995).

Regression-based normalization more comprehensively eliminates variance in hippocampal volume that is associated with intracranial or current brain tissue volumes, compared with proportional percentile- or ratio-based corrections (Free et al., 1995; Van Petten, 2004; Arndt et al., 1991). Rather than creating new variables reflecting 'left/right hippocampal volumes residualized for intracranial/total brain tissue volumes', where appropriate analyses included intracranial/total brain tissue volumes as model covariates in addition to gender and age. This is preferable because it also adjusts for any effects that gender and age may have on associations between hippocampal and intracranial/total brain tissue volumes (Garcia-Berthou, 2001).

5.6. Results

5.6.1. Descriptive statistics

Of the 1091 total LBC1936 participants, 866 attended Waves 1 and 2. Of those, 700 completed the brain MRI scans. As described in Chapter 2 ('*Methodology'*), Individuals who reported history of dementia, had MMSE scores below 24 or did not complete the MMSE at Wave 2 were excluded. Overall, this left 655 participants which had successfully segmented left and right hippocampal volumes. Of these, 624 and 636 participants had successful genotyping for *APOE* ε and *TOMM40* 523, respectively.

In this specific sample of the LBC1936 'Wave 2' dataset (i.e. with segmented hippocampal volumes, *APOE* or *TOMM40* data etc.), *APOE* had allele frequencies of ε2 = 7.4%, ε3 = 76.9% and ε4 = 15.7%, with genotype frequencies of: ε2/ε2 = 2 (0.3%), ε2/ε3 = 83 (12.0%), ε2/ε4 = 15 (2.2%), ε3/ε3 = 401 (58.2%), ε3/ε4 = 175 (25.4%) and ε4/ε4 = 13

(1.9%). *TOMM40* 523 had allele frequencies of S = 41.0%, L = 15.4% and VL = 43.6%, with genotype frequencies of S/S = 106 (15.2%), S/L = 103 (14.7%), S/VL = 259 (37.1%), L/L = 16 (2.3%), L/VL = 80 (11.4%) and VL/VL = 135 (19.3%). Exact tests with an online calculator (2012) confirmed that *APOE* ε and *TOMM40* 523 genotypes were in Hardy-Weinberg equilibrium (*P* values = 0.449 and 0.111 respectively). Descriptive statistics are shown in Table 5.2, and intercorrelations between intracranial, total brain tissue and hippocampal volumes in mm^3 are presented in Table 5.3, showing medium to strong statistically significant intercorrelations. Reported volumes are similar to those in independent samples (Free et al., 1995).

Table 5.2. Descriptive statistics for raw hippocampal, intracranial and total brain tissue volumes, grouped by genotype.

Genotype (mean; SE)	Left raw hippocampal volume	Right raw hippocampal volume	Intracranial volume	Total brain tissue volume	Left hippocampal volume (ICV-corrected)	Right hippocampal volume (ICV-corrected)	Left hippocampal volume (TBV-corrected)	Right hippocampal volume (TBV-corrected)
APOE ε								
ε4 absent	3094.41	3335.06	1,442,258.32	1,119,764.90	3104.24	3344.71	3100.36	3341.09
	(20.36)	(19.98)	(4985.87)	(4316.47)	(19.46)	(19.10)	(18.95)	(18.48)
ε4 present	3084.16	3317.20	1,468,448.23	1,130,853.17	3061.01	3294.47	3070.30	3302.68
	(31.25)	(30.67)	(7694.48)	(6671.72)	(29.96)	(29.40)	(29.19)	(28.46)
ε2 present	3038.94	3315.15	1,449,976.37	1,127,564.58	3031.03	3308.35	3024.76	3301.68
	(46.42)	(45.79)	(11,760.64)	(10,219.88)	(43.36)	(43.53)	(42.08)	(41.85)
ε3/ε3	3103.94	3337.77	1,440,660.88	1,127,674.58	3105.62	3339.22	3106.96	3340.64
	(21.33)	(21.04)	(5400.47)	(4663.18)	(19.92)	(20.00)	(19.33)	(19.22)
TOMM40								
'523'								
S/S	3135.63	3360.83	1,442,116.02	1,126,441.85	3144.34	3369.50	3133.37	3358.46
	(44.01)	(43.15)	(10,827.43)	(9377.16)	(41.91)	(41.02)	(40.89)	(39.79)
S/L	3174.39	3401.25	1,473,374.04	1,137,288.23	3146.15	3373.14	3154.67	3380.33
	(43.76)	(42.90)	(10,880.91)	(9472.76)	(41.81)	(40.92)	(40.93)	(39.83)
S/VL	3076.52	3331.20	1,436,902.51	1,116,375.69	3093.33	3347.93	3087.99	3342.88
	(27.96)	(27.41)	(6871.98)	(5951.29)	(26.69)	(26.13)	(25.99)	(25.30)
L/L	3104.79	3331.20	1,487,577.11	1,145,780.72	3059.36	3356.94	3066.82	3363.22
	(110.00)	(27.41)	(27,496.05)	(23,812.37)	(104.87)	(102.65)	(102.25)	(99.52)
L/VL	3028.12	3402.16	1,463,850.24	1,127,040.17	3009.19	3243.97	3017.10	3251.47
	(49.65)	(107.85)	(12,244.22)	(10,603.84)	(47.32)	(46.32)	(46.13)	(44.90)
VL/VL	3057.69	3301.85	1,450,239.49	1,119,956.79	3057.88	3302.04	3064.65	3308.92
	(37.24)	(36.51)	(9307.98)	(8060.97)	(35.45)	(34.70)	(34.60)	(33.67)

Note. All volumes are in mm^3, estimated marginal means adjusted for age and gender. ICV-corrected = additionally adjusted for intracranial volume, TBV-corrected = additionally adjusted for total brain tissue volume. S/L/VL = Short/Long/Very-long. SE = standard error.

Table 5.3. Inter-correlations between hippocampal, intracranial and total brain tissue volumes.

	Left hippocampal volume	Right hippocampal volume	Intracranial volume	Total brain tissue volume
Left hippocampal volume	-	0.78	0.40	0.47
Right hippocampal volume	0.78	-	0.45	0.50
Intracranial volume	0.40	0.45	-	0.87
Current brain tissue volume	0.47	0.50	0.87	-

Note. Unadjusted Pearson bivariate correlations. All volumes are raw in mm^3, and figures reflect '*r*' correlations. All associations were significant at $P < 0.001$.

5.6.2. APOE ε, TOMM40 523 poly-T repeat and hippocampal volumes

There was no effect of the *APOE* ε4 present vs. absent comparison (*'Step 1'*), ε3/ε4 vs. ε3/ε3 comparison ('*Step 2'*), nor pooled ε2/ε3; ε2/ε2 genotype (vs. ε3/ε3; '*Step 3'*) on hippocampal volumes analysed raw, or normalized for either intracranial or total brain tissue volumes (all $P > 0.05$; see Table 5.4).

No effects of *TOMM40* 523 poly-T repeat genotype were found in the whole sample ('*Step 1'*), nor in subgroup analyses of *APOE* ε3/ε4 (*'Step 2'*) or ε3/ε3 genotypes specifically ('*Step 3'*) for any of raw or normalized hippocampal volumes (all $P > 0.05$; see Table 5.5).

Table 5.4. Apolipoprotein-e (*APOE*) ε genotype and hippocampal volumes.

Volume in mm^3	ε4 allele presence vs. absence			ε3/ε4 vs. ε3/ε3			ε2/ε3 & ε2/ε2 vs. ε3/ε3		
	d.f. & F statistics	*P*	Partial η^2	d.f. & F statistics	*P*	Partial η^2	d.f. & F statistics	*P*	Partial η^2
Left raw hippocampal volume	1, 620 = 0.08	0.784	0.000	1, 519 = 0.33	0.566	0.001	1, 434 = 1.61	0.205	0.004
Right raw hippocampal volume	1, 620 = 0.24	0.626	0.000	1, 519 = 0.48	0.488	0.001	1, 434 = 0.20	0.654	0.000
Left hippocampal volume (ICV-corrected)	1, 619 = 1.45	0.228	0.002	1, 518 = 2.43	0.120	0.005	1, 433 = 2.43	0.120	0.006
Right hippocampal volume (ICV-corrected)	1, 619 = 2.04	0.154	0.003	1, 518 = 2.92	0.088	0.006	1, 433 = 0.41	0.521	0.001
Left hippocampal volume (TBV-corrected)	1, 618 = 0.75	0.388	0.001	1, 517 = 1.94	0.164	0.004	1, 433 = 3.12	0.077	0.007
Right hippocampal volume (TBV-corrected)	1, 618 = 1.28	0.258	0.002	1, 517 = 2.59	0.108	0.005	1, 433 = 0.71	0.399	0.002

Note. Age at time of testing and gender statistically controlled. ICV-corrected = intracranial volume included as a covariate, TBV-corrected = total brain tissue volume included as a covariate.

Table 5.5. Translocase of outer membrane 40 (*TOMM40*) '*523*' poly-T repeat genotype and hippocampal volumes.

Volume in mm^3	Whole sample			ε3/ε4 genotype subgroup			ε3/ε3 genotype subgroup		
	d.f. & F statistics	*P*	Partial η^2	d.f. & F statistics	*P*	Partial η^2	d.f. & F statistics	*P*	Partial η^2
Left raw hippocampal volume	5, 628 = 1.47	0.198	0.012	1, 156 = 1.42	0.235	0.009	2, 349 = 1.32	0.268	0.008
Right raw hippocampal volume	5, 628 = 1.23	0.292	0.010	1, 156 = 0.61	0.435	0.004	2, 349 = 1.57	0.210	0.009
Left hippocampal volume (ICV-corrected)	5, 627 = 1.47	0.198	0.012	1, 155 = 1.22	0.271	0.008	2, 348 = 1.15	0.316	0.007
Right hippocampal volume (ICV-corrected)	5, 627 = 1.33	0.250	0.010	1, 155 = 0.429	0.513	0.003	2, 348 = 1.70	0.184	0.010
Left hippocampal volume (TBV-corrected)	5, 626 = 1.34	0.244	0.011	1, 154 = 1.55	0.216	0.010	2, 348 = 0.79	0.455	0.005
Right hippocampal volume (TBV-corrected)	5, 626 = 1.18	0.319	0.009	1, 154 = 0.67	0.415	0.004	2, 348 = 1.30	0.274	0.007

Note. Age at time of testing and gender statistically controlled. ICV-corrected = intracranial volume included as a covariate, TBV-corrected = total brain tissue volume included as a covariate.

5.6.3. Additional comparative analysis

To permit comparison with other reports that use alternative methods of normalization for head size – namely, reporting hippocampal volumes as an absolute proportion of total intracranial volume – the above main results were re-run using the formula "Left-or-right hippocampal volume in mm^3 / intracranial volume in mm^3 x 1000" (DeToledo-Morell et al., 2004). Re-analysis showed that the main results were for the most part unchanged, i.e. not significant at $P < 0.05$. One exception was that for the *APOE* ε4 present vs. absent comparison, there was a significant deleterious effect of the ε4 allele for the right hippocampus ($F [1, 620] = 4.54$, $P = 0.034$, $\eta^2 = 0.007$).

To check the extent to which left and right hippocampal 'ratios' were independent of intracranial volume, unadjusted bivariate correlations were run. Intracranial volume correlated significantly with left ($r = -0.26$) and right ($r = -0.28$) hippocampal volumes expressed as a proportion of intracranial volume (both $P < 0.001$), indicating a lack of true independence.

5.7. Discussion

5.7.1. Overview

The current study investigated the effects of the *APOE* ε and *TOMM40* rs10524523 ('*523)*' poly-T repeat gene loci on hippocampal volumes - raw and also corrected (commonly and herein referred to as 'normalized') separately for intracranial and total brain tissue volumes - in the LBC1936. This normalization was implemented because analyses aimed to test for genetic influence on the hippocampus independent of any possible effects on intracranial or total brain tissue volumes. This study reports no significant effects of genetic variation at

either the *APOE* ε or *TOMM40* 523 loci, on left or right hippocampal volumes, when analysed either raw or normalized by intracranial or total brain tissue volumes.

Hippocampal volumes and *APOE* have been investigated in a number of previous reports, the majority of which were relatively small (N < 135). *TOMM40* 523 and hippocampal volumes have been investigated by one previous study (Johnson et al., 2011), however that report examined specifically participants with the *APOE* ε3/ε3 genotype (N = 117), and found no significant associations. The current study tested the *TOMM40 523* locus in a larger sample of older adults and included all *APOE* ε genotypes (n = 623), ε3/ε4 carriers only (n = 160), and ε3/ε3 carriers only (n = 376), and found no effect of poly-T repeat length genotype.

5.7.2. Interpretation: APOE ε

Previous large studies have reported significant effects of *APOE* ε genotype on hippocampal volumes in healthy older adults (LeMaitre et al., 2005; Den Heijer et al., 2002). In a similarly large sample of older adults, this study did not replicate these, and instead echo other large reports which show no association with genetic variation at the *APOE* locus (Cherbuin et al., 2008; Panizzon et al., 2010; Ferencz et al., 2013). There is no evidence that large positive studies by LeMaitre et al. (2005; mean age = 69.2; mean MMSE = 27.3) or Den Heijer et al. (2002; mean age = 72.0 years; mean MMSE score = 27.4) were composed of markedly more cognitively impaired or younger/older subjects than those reported here (mean age = 72.7, mean MMSE = 28.8). The discrepancy may reflect type 1 error in previous reports. Part of the discrepancy may also relate to normalization technique. Previous studies vary in how they normalize left and right hippocampal volumes - namely they use either 'ratio' (e.g. Lemaitre et al.; 2005) or 'covariance' techniques (e.g. Den Heijer et al*., 2002*). Above in the main

results report on hippocampal volumes covaried separately for intracranial and brain tissue volumes as this would seem to be the most appropriate correction (see below; Free et al., 1995; Van Petten, 2004; Arndt et al., 1991).

To permit comparison with other reports, the main results were additionally re-analysed with hippocampal volumes normalized as a ratio of intracranial volume. The results were unchanged except for a significant deleterious effect of *APOE* ε4 allele presence vs. absence. However, statistically controlling for intracranial volume is judged to be the more appropriate normalization technique for the following reasons:

1. Ratio measures do not completely eliminate association between head size and hippocampal volume (Free et al., 1995; Van Petten, 2004; Arndt et al., 1991). Further analysis showed that hippocampal volumes expressed as a ratio of intracranial volume correlated significantly with intracranial volume itself. Ratios therefore do not allow for an assessment of *APOE*, *TOMM40* and hippocampal volume completely independent of head size, which was the main aim of the current study.
2. Any disparity between genotype groups as assessed by ratios may reflect differences in any of the numerator (hippocampus), the denominator (intracranial volume) or their interaction, reduced to one variable, which could lead to spurious type 1 error. This variable is generally less informative than regression techniques which take into account the strength of association between the specific brain structure and the larger denominator (Free et al., 1995; Arndt et al., 1991)

Critically reviewing previous reports further, there appears to be no study that corrects significant *APOE*-hippocampal volume associations for current total brain tissue volume. MacLullich et al. (2002) reported in a sample of older adults (N = 97, age range = 65-70 years), that left/right hippocampal, frontal lobe, temporal lobe and intracranial volumes strongly and positively intercorrelated with one another (r range = 0.29 to 0.83, all $P <$

0.005). Data reduction with principal component analysis showed that these loaded strongly and significantly onto a 'general brain size' factor (range of loadings = 0.64 to 0.73, $P < 0.05$). Intracranial volume is relatively constant throughout the lifespan, while actual brain tissue volume is susceptible to age-related change (Rushton et al., 1996). Given that the author is not aware of any previous study that reports significant *APOE*-hippocampal volume associations in older adults that also controls for current total brain tissue volume, we cannot exclude the possibility that those associations may be secondary to more generalized brain atrophy (Den Heijer et al., 2002; MacLullich et al., 2002; Shenkin et al., 2009; Raji et al., 2012). (Note however that Den Heijer et al. reported no significant ε4 effect on semi-qualitative observer-rated cortical atrophy; ranged 0-3 at various locations). It is also unclear whether previous large significant positive studies have assumed normal distributions for normalized hippocampal volumes in older-age samples, as this may introduce errors in analysis and increase the risk of spurious results.

5.7.3. Interpretation: TOMM40 '523' poly-T repeat

No effects of *TOMM40* 523 were found, and this could be cautiously interpreted in terms of different explanations, given the absence of further relevant data. Specifically:

1. The *TOMM40 523* repeat does not significantly affect mitochondrial function (Cruchaga et al., 2011; Bekris et al., 2012; Hedskog et al., 2012).
2. *TOMM40* 523 locus does affect mitochondrial function, but not to an extent that affects hippocampal volume (the outcome variable) in this sample: the mitochondrial cascade hypothesis describes a 'threshold' beyond which mitochondrial mutations are not adequately compensated for and significant histology resembling AD emerges (Braak and Braak, 1995; Ohm et al., 1999). Perhaps hippocampal volume assessed by MRI is unaffected before this threshold (Swerdlow and Kahn, 2004).

3. The effect of *TOMM40* 523 length is moderated by or interacts with additional genetic variants for example loci in LD with *APOE* or *TOMM40* such as *APOC1* (Bekris et al., 2012). It may also be possible to investigate the possibility of moderation of *TOMM40*/*APOE* effects by other genetic factors; however, despite the relatively large sample size (by brain imaging standards), this would be statistically challenging.

5.7.4. Limitations and future research

This study examined left and right hippocampal volumes; specific subregions of the hippocampal formation may be more vulnerable to brain ageing or incipient AD pathology, and therefore more sensitive to variations at specific relevant genetic loci. Devanand et al.(2012) reported that subregions of the hippocampus differentially predicted longitudinal diagnosis of clinical AD over three years, in a sample of individuals with amnestic mild cognitive impairment (N = 130, of which 31 converted to AD; baseline MMSE scores all >22). Controlling for age, gender, years of education, and intracranial volume, cox regression analyses showed that volumes in the cornu ammonis 1 (left hazard ratio = 0.22, P = 0.054, right hazard ratio = 0.23, P = 0.06) and subiculum subregions (left hazard ratio = 0.22, P = 0.054, right hazard ratio = 0.22, P = 0.03) were more predictive of longitudinal AD diagnosis compared with entorhinal cortical volume (right hazard ratio = 0.05, P = 0.06, left hazard ratio = 0.02, P = 0.26). Future studies of hippocampal volume in the LBC1936 may therefore consider more fine-grained analysis of the hippocampal formation as genetic variation at the *APOE* or *TOMM40* loci may affect specific subregions first. Functional brain MRI may also be a more sensitive marker of hippocampal dysfunction, compared with left/right volumes as assessed here by structural MRI.

It is possible that the current sample of non-demented, generally healthy older adults (aged around 73 years) have not undergone sufficient volumetric hippocampal or brain

atrophy to show significant differentiation according to genotype. The sample examined here is undergoing repeat structural brain MRI, around the age of 76. Significant associations with *APOE* or *TOMM40* genotypes may become apparent at this older age, after more age-related atrophy.

5.7.5. Summary

This study examined the independent effects of variation at the *APOE* ε and the *TOMM40 523* poly-T repeat gene loci upon hippocampal volume assessed raw and also normalized separately for intracranial and total brain tissue volumes. Previous large studies have occasionally reported significant associations between the *APOE* ε4 allele and lower hippocampal volumes. The current study does not replicate those significant reports in a community dwelling sample of older adults of homogenous ages. Previous significant findings may reflect type 1 error, or partly reflect discrepancies in how the hippocampus has been normalized relative to head size in different studies. There appears to be no obvious evidence that previously examined healthy older samples differed markedly from the current sample, either in terms of age or prevalence of cognitive decline (based on average MMSE scores). Studies that show significant genetic associations with the hippocampus should run further confirmatory analysis to investigate whether associations are independent of general volumetric brain atrophy. In this study there were also no significant effects of *TOMM40* 523 poly-T repeat length. Future studies may investigate specific subregions of the hippocampal formation; there may be effects of the *APOE* or *TOMM40 523* genetic loci that are not manifest in overall hippocampal volume.

Chapter 6: The *APOE* ε and *TOMM40* poly-T repeat genetic loci, white matter lesions and cerebral microbleeds

A version of this chapter is in preparation for submission for publication*:* Lyall, D.M., Valdés Hernández, M. del C., Harris, S.E., Royle, N.A., Bastin, M.E., Muñoz Maniega, S., Murray, C., Lutz, M.W., Saunders, A.M., Roses, A.D., Starr, J.M., Porteous, D.J., Deary, I.J. & Wardlaw, J.M.

6.1. Abstract

Two purported brain imaging markers of cerebral small vessel disease are white matter lesions and cerebral microbleeds, and these are significantly more common in people with Alzheimer's disease (AD). Variations in the *APOE* ε haplotype and *TOMM40* rs10524523 ('523') variable length poly-T repeat have previously been significantly associated with AD, and this study sought to determine if these variations also associated with white matter lesion and microbleed burden in community-dwelling, generally healthy older adults.

As part of a longitudinal study of cognitive ageing, participants in the Lothian Birth Cohort 1936 underwent genotyping for *APOE* ε2/ε3/ε4 status and *TOMM40* 523 poly-T repeat length, and detailed structural brain MRI at a mean age of 72.70 years (standard deviation [SD] = 0.74, n = ~700).

No significant effects of *APOE* ε or *TOMM40* 523 genotype were found on white matter lesion burden (assessed volumetrically and also semi-qualitatively with the Fazekas scale), or cerebral microbleed burden (assessed with the Brain Observer MicroBleed Scale).

In a large community-dwelling sample of non-demented older adults, analyses found no significant effects of *APOE* ε or *TOMM40* 523 genotypes on the intermediate phenotypes of brain white matter lesions or cerebral microbleeds. This is discrepant with some previous reports of significant association between *APOE* and both of these phenotypes, however generally supports other null studies and recent reviews which conclude that any association is modest at best. Previous significant findings may partly reflect type 1 error, or be due to differences in the age or prevalence of cardiovascular/cerebrovascular pathology in particular samples.

6.2. Introduction

There is evidence that the presence of cardiovascular disease pathology can increase the future risk of Alzheimer's disease (AD) and cognitive decline. Two genetic risk factors are in the *APOE* and *TOMM40* gene loci, and these have been occasionally associated with cognitive ability and AD-related phenotypes such as age of onset. Two specific brain MRI phenotypes – namely white matter lesions and brain microbleeds – are purported markers of cerebral small vessel disease. The present study tests for association between *APOE* and *TOMM40*, separately with white matter lesions and cerebral microbleeds. Having described the *APOE* ε and *TOMM40* '523' poly-T repeat gene loci in Chapter 1 ('*Introduction'*) and Chapter 2 ('*Methodology'*), the following sections of the introduction detail cardiovascular disease burden and its relevance to AD/cognitive decline, then white matter lesions, and then cerebral microbleeds and their relevance to AD/cognitive decline. Finally, previous studies of *APOE* ε/*TOMM40* 523 genetic contributions to white matter lesions/microbleeds are described.

6.2.1. Cardiovascular disease burden, Alzheimer's disease and cognitive decline

Large-scale reports indicate significant positive association between possession of cardiovascular disease pathology, such as diagnosis of high blood pressure or diabetes, and increased risk of dementia and/or cognitive decline in the future (Launer, 2002; Brickmann et al., 2012; Debette and Markus, 2010; Lo et al., 2012; examples detailed below).

The precise mechanisms underlying significant association between cardiovascular and neurodegenerative pathology are unclear, however there are three main hypotheses (Stampfer, 2006). Firstly, it is possible that cardiovascular diseases and AD/cognitive decline share common risk factors and are not mechanistically related (Stampfer, 2006). Secondly, it is possible that cardiovascular burden may expedite progression of AD/cognitive decline

through promoting atherosclerosis and accumulations of amyloid-beta plaques, and/or (thirdly) by increasing vulnerability to such pathology and lowering the threshold at which cognitive decline becomes apparent behaviourally, even in the absence of a mechanistic link (Stampfer, 2006; Brickmann et al., 2011; Snowden et al., 1997). Two studies are described provided below (in turn) which report that baseline cardiovascular disease increased risk of AD and cognitive decline, in relatively large samples:

Kivipelto et al. (2001) reported that midlife cerebrovascular risk factors associated significantly and positively with risk of AD in later life. Participants from Finland received detailed physical examinations in midlife (n = 1409; mean age = 50.4 years, SD = 6.0), and again in later life (mean age 71.3, SD = 4.0) by which point 48 participants had developed AD, while 1352 had not. Midlife cerebrovascular risk factors significantly and positively increase the risk of AD in later life, including high systolic blood pressure (i.e. ≥159 mmHg; odds ratio [OR] = 3.1, 95% C.I's 1.4 to 6.6), diastolic blood pressure (i.e. ≥95 mmHg; OR = 1.7, 95% C.I's 0.88 to 3.2) and overall cholesterol concentrations (≥65 mmol/l; OR = 2.9, 95% C.I's 1.4 to 4.4). Having combined high systolic blood pressure and high overall cholesterol was associated with a marked increase in risk (OR = 3.3, 95% C.I's 1.7 to 6.4).

Dregan et al. (2012) examined a large prospective sample; the English Longitudinal Sample of Ageing. Participants received cognitive and physical assessments (including blood pressure, cholesterol, body mass index, mood, smoking/drinking/exercise history etc.) at three time points (1998-2001, 2004-2005 and 2008-2009). Mean age at baseline was 62.5 (no age-range information was provided; N = 11,205). Participants completed cognitive tests from executive, memory, reasoning and memory domains, and scores were summed to create general 'cognitive indexes'. At 2008/2009, 6,260 participants remained. Controlling for education, alcohol intake, physical exercise, depressive symptoms and mood, Dregan et al. found that baseline high systolic blood pressure (≥160 mmHg; unstandardized β = -1.26, 95%

C.I's = -2.52 to -0.01) and history of smoking (unstandardized β = -1.51, 95% C.I's = -2.29 to -0.074) were both associated with significantly lower cognitive index scores in 2008-2009.

White matter lesions and cerebral brain microbleeds are generally considered to reflect cerebrovascular burden in ageing; they are manifestations and markers of cerebral small-vessel disease (Charidimou and Wearing, 2012). White matter lesions are relatively common in normal human ageing (Debette and Markus, 2010) and reflect increased water content relative to fatty lipid white matter; this may reflect demyelination, gliosis, axonal loss or possible infarction (Wahlund et al., 2001; Barkhof and Scheltens, 2002). Brain microbleeds are small haemosiderin deposits, possibly indicative of past cerebral amyloid angiography or hypertensive angiopathy linked to arteriosclerosis (Greenberg et al., 2009; Charidimou and Werring, 2012). (Haemosiderin is a remnant product from white blood cells engulfing the haemoglobin from decayed red blood cells after a haemorrhage).

White matter lesions and brain microbleeds often co-occur; Wardlaw et al. (2006) for example reported that microbleeds were significantly more common in Stroke patients with greater white matter lesion pathology (N = 308, median age = 66.0 years, range = 19-89; ≥1 microbleeds found in 54% of patients with Fazekas rating-scale scores ≥ 3 vs. 8% for those with scores of 0; $P < 0.001$). It is possible that these pathologies reflect diffuse abnormality of small cerebral microvessels and arterioles, including microatheroma, vasospasm, or arterial wall thickening, and subsequently impaired auto-regulation which leads to ischemia (Wardlaw, 2005). While matter lesions are more commonly considered to have ischemic origin, while microbleed pathology may relate more to microvascular permeability (i.e. leakage of the blood brain barrier; Wardlaw et al., 2003). The exact mechanisms are unclear, however (Fazekas and Wardlaw, 2013). These brain MRI phenotypes – brain white matter lesions and microbleeds - are detailed hereafter in turn.

6.2.2. White matter lesions

Human brain white matter is characterised by a fatty myelin layer that facilitates the high-speed processing of information (Penke et al., 2012). White matter lesions can be assessed by brain MRI scans, namely T2-weighted or fluid attenuated inversion recovery (FLAIR) images. Lesions appear as high-intensity signals on scans (often 'hyper intensities'). Typically these hyperintensities appear in white matter confluent to the lateral ventricles (commonly 'periventricular'), or deep within cortical white matter (commonly 'deep').

De La Torre (2010) refers to the link between cardiovascular and brain function as 'neurovascular coupling' in reference to the cardiovascular system dynamically providing blood to the brain. Cerebral white matter is largely supplied by long penetrating arteries and arterioles, the obstruction of which is associated with ischemia and white matter lesions (Assareh et al., 2010). White matter lesions are therefore purportedly a brain metric reflecting cerebral small vessel disease, in particular ischemia (Schuur et al., 2011; Brickman et al., 2009).

White matter lesions are important because they increase the risk of stroke, cognitive decline and diagnosis of dementia. Debette and Markus (2010) systematically reviewed primary studies published between 1966 and 2009 that had baseline white matter lesion burden as well as longitudinal data ranging from 1.3 to 9.5 years follow-up in terms of medical history. These studies variously tested the predictive power of lesion burden on longitudinal prospective a) incident stroke (6 healthy samples, 3 non-healthy/high risk samples e.g. diabetic), b) dementia (respectively 3 healthy, 3 non-healthy), and c) cognitive decline (5 healthy only). Cognitive decline was defined as a marked reduction in scores on cognitive screening tools, or general cognitive ability as assessed with specific tests of different domains such as memory. In terms of white matter lesions, all participants were assigned into 'high' or 'low burden' at baseline. Sample mean ages in different studies

ranged from 60.2 to 80.1 years at baseline. In terms of white matter burden and longitudinal risk of incident stroke, a meta-analysis including healthy and non-healthy samples showed a significant and positive association (total sample N = 13,539; OR = 3.5, P <0.001). Greater white matter lesion burden was also significantly associated with greater longitudinal risk of dementia (sample N = 8803; OR = 1.9, *P* = 0.002), and cognitive decline (OR = 2.0, P not reported). The predictive power of baseline white matter lesion burden on longitudinal dementia/cognitive decline risk may therefore reflect cerebrovascular damage on the route to AD (i.e. it is a marker of progression but does not does not necessarily affect/interact), or it may affect/interact with other pathological changes to accelerate expression (Debette and Markus 2010; Brickman et al., 2012).

6.2.3. Cerebral microbleeds

Cerebral microbleeds appear as round or oval hypointense signals on T2*-weighted MRI (Greenberg et al., 2009). These reflect small deposits of blood product (haemosiderin) that typically appear at under 10 millimetres in diameter; microbleed size appears relatively bimodal around 0-5 mm and 5-10 mm, however because studies often differ in specific MRI pulse sequences, specific size is considered less important than frequency and - more importantly - location (Greenberg et al., 2009).

In terms of frequency, microbleeds increase in older age and in the presence of cardiovascular diseases (Maxwell et al., 2011). In a large review of primary studies that examined cerebral microbleeds, Cordonnier et al. (2007) reported a prevalence of 5% in healthy older participants (total N = 1411), compared with 33.5% in adults with history of ischemic stroke (N = 1075), and 60.4% in participants with history of nontraumatic intracerebral haemorrhage (N = 894).

Microbleeds in different locations in the brain appear to reflect different underlying pathophysiologies. Microbleeds in strictly lobar regions (e.g. cortex/grey-white matter junction; subcortical white matter) are interpreted as reflecting underlying cerebral amyloid angiopathy while infratentorial (e.g. basal ganglia; internal/external capsule; thalamus) or cerebellar (e.g. brainstem; cerebellum) areas are possibly more reflective of hypertensive/ischemic vasculopathy (Viswanathan et al., 2010; Greenberg et al., 2009; Cordonnier et al., 2011). As an example, Vernooij et al. (2008) examined a large sample of generally healthy older adults (n = 1062, mean age = 69.9, SD = 7.2). Participants underwent detailed MRI, genotyping and medical testing. They found that prevalence of hypertension (non; mild; severe) was additively associated with deep or infratentorial microbleeds only (severe vs. non-hypertensive OR = 1.66, 95% C.I's 0.91 to 3.05), but not strictly lobar microbleeds. In contrast, the association between *APOE* ε4 'risk' allele presence (generally associated with greater increased amyloid-β depositions and risk of cerebral amyloid angiography; vs. ε3/ε3) was stronger for lobar (OR = 1.87, 95% C.I's = 1.25 to 2.81) compared with deep/infratentorial microbleeds (OR = 1.17, 95% C.I's = 0.70 to 1.93).

Microbleeds are significantly more prevalent in individuals with AD – primarily but not exclusively in lobar regions of the brain (Greenberg et al., 2009). Cordonnier et al. (2011) report microbleed frequencies in a collated set of data from 5 small studies that examined microbleeds in individuals diagnosed with AD (all sample n's examined under 80 people except for one sample n = 223). They found prevalence rates of 23% (95% C.I's = 17 to 31%), much higher than would be expected in relatively healthy older adults.

Microbleeds may relate to cognitive decline by affecting brain tissue at a specific location (i.e. playing a mechanistic role), and/or by may be reflective of underlying cerebrovascular disease burden, which is in reality the true mechanism affecting mental ability (Greenberg et al., 2009). Poels et al. (2011) examined a large sample of generally

healthy older adults in terms of cognitive ability and microbleeds (n = 3979, mean age = 60.3, SD = 8.7). They analysed microbleed frequency in terms of 0 vs. 1 vs. 2-4 vs. ≥5, and split location by strictly lobar vs. deep/intratentorial. Presence (i.e. ≥1) of strictly lobar cerebral microbleeds was associated with significantly lower performance on a general factor of information processing speed (standardised β = -0.03, 95% C.I's -0.06 to -0.01) and motor speed (standardised β = -0.04, 95% C.I's -0.08 to -0.01). Presence of deep/infratentorial cerebral microbleeds was associated with lower cognitive ability on various domains only for the ≥5 vs. 0 comparison. Only the strictly lobar-speed associations survived correction for education and cerebrovascular risk factors such as systolic/diastolic blood pressure, smoking history, diabetes total cholesterols, and use of lipid/pressure related drugs; this may suggest an independent mechanistic disruption of brain functional connectivity.

6.2.4. APOE, TOMM40 & white matter lesions/cerebral microbleeds

Previous studies have examined *APOE* and white matter lesions. Paternoster et al. (2009) examined all primary studies reported between 1966 to the end of 2007, including healthy and non-healthy individuals. They extracted different white matter hyperintensity statistics (e.g. periventricular/deep/overall lesions) and examined burden three ways: 1) by splitting participants into high or low lesion burden groups, based on semi-qualitative scores, 2) by raw semi-qualitative scores (e.g. Fazekas scores 0 to 4), and 3) volumetrically assessed in cm^3/mm^3/ml adjusted for head size. Samples were split into healthy and non-healthy (e.g. diagnosed with hypertension), and also collated, as separate analyses. There were 24 studies reviewed in total (n = 8564), of which two included non-healthy participants (n = 96). Meta-analysis was conducted on 18 samples (n = 3465) and showed no significant association between *APOE* ε and membership of the high-lesion group (total OR = 0.97, 95% C.I's 0.78 to 1.21), graded burden (OR = 0.30, 95% C.I.'s -0.02 to 0.62), or volumetric burden (OR =

0.15, 95% C.I.'s -0.04 to 0.33). Of the studies that could not be included in the meta-analysis because of unavailable data, none showed statistically significant associations (Paternoster et al., 2009). It should be noted that of the 24 studies examined, only 8 included relatively healthy older adults and of those only 5 were included in the meta-analysis (n = 1176). More recent studies in healthy older adults have been conducted since end of 2007. See Table 6.1 for a collated list of studies in community-dwelling older adults. Observing this table, there is a generally inconsistent association between ε4 genotype and white matter lesion burden. No studies appeared to correct for multiple testing.

A large review of *APOE* and cerebral microbleeds was conducted by Maxwell et al. (2011). They examined 10 primary articles totalling 7,351 adults (3 healthy samples, n = 5977; 7 with neurological conditions n = 1374). Mean ages ranged from 45 to 76 years. Of these 10 studies, 7 assessed microbleeds as dichotomous present vs. absent variables, one measured frequency and two were entirely qualitative. The seven with dichotomous microbleed data (including healthy and neurological samples) were meta-analysed (n =7272, number with ≥1 microbleed = 1156; overall mean age = 66.7 years). When they compared ε4+ with ε3/ε3, they found a significant increase in the presence of at least one microbleed (OR = 1.22, 95% C.I's = 1.05 to 1.41, P = 0.01). The association was slightly stronger for strictly lobar microbleeds (OR = 1.35, 95% C.I's = 1.10 to 1.66, P = 0.005) compared with not strictly lobar (OR = 1.16, 95% C.I's = 0.89 to 1.50, $P > 0.05$) (vs. no microbleeds). Notably, only four of seven studies adjusted for cerebrovascular risk factors. To date, four large studies have been conducted examining *APOE*, cerebral microbleeds in healthy older adults (examining three samples; See Table 6.2). Observing table 6.2, it is apparent that significant associations with ε4 are either a) for the rare ε4/ε4 genotype vs. ε3/ε3, or b) in a sample with slightly high degree of cardiovascular pathology and smoking prevalence.

Table 6.1. Summary of previous studies analysing apolipoprotein-e and white matter lesions in non-demented, community dwelling older adults.

Report	*Imaging; measures*	*Sample (mean age; SD)*	*N*	*Covariates*	*Phenotypic association (direction of ε4)*	*Notes & limitations*
Schmidt et al. 1996*	Fazekas scale: dichotomous high/low burden (Peri. + deep).	Healthy older adults (~52 years; SD unclear).	214	Unavailable.	Odds ratio = 0.88 (95% C.I.'s = 0.34 - 2.28).	Specific data could not be retrieved (covariates etc).
Kuller et al., 1998	Fazekas scale: graded 0-9 (Peri. + deep).	Healthy older adults (>65; SD unclear).	3480	Age, sex, race, education, clinical disease history. (n.s.)	Odds ratio = 1.4 (95% C.I's = 1.1 to 1.9).	
Skoog et al., 1998*	Graded dichtotomous high/low burden (Peri. + deep).	Healthy older adults (85 to 88; SD unclear).	117	None apparent.	Odds ratio = 0.89 (95% C.I.'s = 0.41 to 1.92).	Used CT, which is less detailed than MRI.
DeCarli et al., 1999*	Volumetric (continuous in mm^3).	Healthy/CVD older adult twins (72.6; ±3.0**).	396	Age, education, ICV, cardiovascular disease history. (Statistically controlled).	Reported significant effects of ε4 in diseased sample only (ε4+ = 12cm^3 vs. e4- = 7 cm^3; P <0.05). No effects in healthy people.	Sample of twins. Did not adjust for relatedness.
Nebes et al., 2001*	Graded dichotomous (high/low burden; deep only).	Healthy older adults (73.6; ±3.4).	92	Education. (Controlled).	Odds ratio = 1.40 (95% C.I.'s = 0.52 to 3.72).	Sample of mixed races. Highly educated (mean = 16.3 years).
Bigler et al., 2003*	Graded dichotomous (high/low burden; deep only).	Healthy older adults (~70; data could not be retrieved).	215	Data could not be retrieved.	Data could not be retrieved. Report no significant effect of *APOE*.	Data could not be retrieved.

Continued

Continued

De Leeuw et al., 2004*	Continuous volumes (deep + perventricular).	Healthy older adults (72.1; ±7.3**).	829	Age, sex, cardiovascular risk factors; BMI, peripheral arterial disease, diabetes, study site. (Controlled).	Main effect: adjusted mean difference for ε4 carriers = 0.5 milliletres, 95% C.I's = 0.2 to 0.8).	Significant interaction with hypertensive status, where ε4+hypertension had greatest periventricular lesion load (see text).
Hogh et al., 2007	Graded (Scheltens scale; peri. + deep).	Healthy older adults (82.3; ±0.8).	75	Cardiovascular risk factors such as smoking and diabetes (n.s.).	Significantly increased burden in overall white matter: ε4 effect (F = 5.72, *P* = 0.02).	No interaction with risk drinking, gender and hypertensive status.
Raz et al., 2012	Continuous overall volume.	Healthy older adults (58.9; ±9.09).	144	Hypertension, age, gender, ICV. (Controlled).	Lobar x genotype interaction: (F [6, 414] = 3.29, *P* = 0.006; data unavailable).	No ε4 effect.
Hafsteinsdottir et al., 2012	Continuous overall volume.	Healthy older adults (76.0; ±5.4**).	4303	ICV, age, gender, BMI, education, mood history. (Controlled).	Significant effect: ε4+ = 0.98 cm^3 vs. ε4- = 0.95 cm3 (*P* < 0.01).	Large sample.
Schuur et al., 2011	Continuous overall volume.	Older hypertensive adults with (64.7; ±4.3**).	139	Age, gender, total ICV. (Controlled).	No significant effects of *APOE* genotype.	
Godin et al., 2009	Continuous volume: cross sectional and longitudinal ~4 years later.	Healthy older adults (72.4; ±4.1).	1779 baseline; 1319 longit.	Education, total ICV, gender, age. (Controlled).	Baseline: effects of ε4/ε4 vs. ε4/- vs. -/- (5.58 vs. 5.34 vs. 8.73 cm^3; *P* = 0.004). Longitudinal: similar effects for ε4/ε4 vs. ε4/- vs. -/- (1.32 vs. 1.48 vs. 3.46 cm3 change respectively, *P* = 0.01).	Significantly greater effect of ε4 homozygosity.

* = included in Paternoster et al. (2009) meta-analysis. Peri. = periventricular white matter lesions, SD = standard deviation, ICV = intracranial volume. ** = weighted estimates.

Table 6.2. Summary of previous studies analysing apolipoprotein-e and cerebral microbleeds in non-demented, community dwelling older adults.

Report	*Imaging; measures*	*Sample (mean age; SD)*	*n*	*Covariates*	*Phenotypic association (direction of* ε4)	*Notes & limitations*
Sveinbjornsdottir et al., 2008	Cerebral microbleeds assessed by ≥2 independent raters. Presence vs. absence (any area). Prevalence = 11.1%.	AGES-Reykjavik study; healthy older adults (76.0; ±6.0).	1962	Age, gender, hypertension, diabetes, smoking history, self-reported history of stroke/transient ischaemic attach. (n.s.).	Significant effect of ε4/ε4 genotype (≥1 microbleed in 22.5%; vs. ε3/ε3 genotype prevalence = 10.9% (n = 1215).	Does not state hardy-Weinberg statistics. Homozygosity effect only.
Jeerakathil et al., 2004	Cerebral microbleeds assessed by ≥2 independent raters. Presence vs. absence (any area). Prevalence = 4.7%.	Framingham Study Original Cohort and Offspring Cohort; healthy older adults (63.9; ±12.0).	368	Systolic blood pressure, total cholesterol, high-density lipoprotein, age, smoking status, diabetes, gender. (n.s.).	No significant effect of ε4 (P = 0.316). *APOE* ε4/ε4 frequencies not reported.	Only moderate inter-rater agreement based on 222 scans read (0.33 to 0.57). Does not state hardy-Weinberg statistics.
Vernooij et al., 2008	Cerebral microbleeds assessed by ≥2 independent raters. Presence vs. absence (any; deep; infrantentorial; lobar). Prevalence = 29.1%.	Rotterdam Study; healthy older adults (69.6; ±7.2).	1062	Blood pressure; hypertensive status, smoking habits, alcohol use, diabetes, serum total/HDL cholesterol, use of lipid lowering and blood pressure lowering medication. (n.s.).	Significant effect of ε4 allele; (odds ratio = 1.87, 95% C.I's = 1.25 to 2.81 ; vs ε3/ε3). More pronounced effect for lobar only (odds ratio = 2.68; 95% C.I's = 1.37 to 5.27). *APOE* ε4/ε4 frequencies not reported.	High proportions of cardiovascular disease history, e.g. 51.5% with mild hypertension, 20% with severe hypertension, 72% ever-smokers.

Continued

Continued

Poels et al., 2011a	Longitudinal onset of new microbleeds since above study. Presence vs. absence (any; deep; infrantentorial; lobar).	Rotterdam Study; healthy older adults (68.5; ±6.3).	831	Age, sex, scan interval (from above study). (Controlled). Similar cardiovascular covariates as above.	ε4/ε4 group showed significant increase in microbleeds (odds ratio = 4.43, 95% C.I's = 1.90 to 22.89; vs. ε3/ε3).	Similar caveats as above.

In general, it would appear that any association between the *APOE* ε locus and white matter lesion/cerebral microbleed burden is modest (Paternoster et al., 2009; Maxwell et al., 2011). However there are important caveats to this based on tables 6.1 and 6.2:

1 Relatively small sample sizes. For example, in a review of white matter lesion studies, Paternoster et al. found that previous population n's ranged from 29 to 427, excluding one study with 944 participants. Of the 18 samples included in meta-analysis, only four had >150 participants. Of the more recent studies not reviewed by Paternoster et al., only one out of four had >150 participants. A large study of independent data would therefore add significantly to the literature. (In the case of microbleeds, there are only four studies conducted, including three independent samples).

2 Wide age ranges: the mean age of studies examining generally healthy older adults in tables 6.1 and 6.2 are relatively wide. Statistically controlling for this variable is unlikely to control for the accumulated effects of all subtle age-related changes, and samples with homogenous age ranges are preferable (Hofer and Sliwinski, 2001).

3 Large studies do not consistently adjust for, or consider genetic interactions with, the effects of cardiovascular pathology on white matter lesion burden or microbleeds. For example, De Leeuw et al. (2004) examined 829 older adults (mean age = 72.1, SD = 7.3) with brain MRI and APOE information. They grouped participants according to normo- vs. hyper-tensive (systolic blood pressure >160 mmHg, diastolic >95 mmHg) at the first scan. White matter lesion burden was rated semi-qualitatively from 0 to 9. Controlling for the effects of age, gender, and other intermediate vascular risk factors such as diabetes, presence of hypertension was associated with significantly increased subcortical (adjusted mean score difference = 1.0, 95% CI's = 0.8 to 1.2) and periventricular (difference = 0.9, 95% CI's = 0.7 to 1.1; both $P < 0.05$) lesion scores, independent of *APOE*. They found a significant interaction where presence of the ε4 allele and hypertension was

synergistically associated with greater burden (interaction $P = 0.016$). An interaction with hypertension therefore seems an important aspect to consider. In addition, other large studies have reported a significant deleterious effect of hypertension on white matter lesion burden in older adults (e.g. van Dijk et al., 2004; N = 1625, mean age = 70.0, SD = 3.0), although not in the context of *APOE*. Hypertension may affect blood-brain barrier permeability and contribute to microvascular instability (van Dijk et al., 2004).

It would seem that no previous study has examined the *TOMM40* poly-T repeat locus and association with white matter lesion or cerebral microbleeds burden. Furthermore, given the inconsistent association between *APOE* and white matter lesions/cerebral microbleeds. A large study with homogenous age ranges that takes cardiovascular disease history into account would contribute significantly to the literature.

6.2.5. Current study

This report aims to investigate the *APOE* ε and *TOMM40* poly-T repeat genetic loci in terms of white matter hyperintensity burden in a large sample of community-dwelling older adults. This will add significant data to the literature in terms of 1) a large set of additional well-controlled data with a narrow age range, analysed in terms of *APOE* genotype, 2) novel investigation of the *TOMM40* poly-T repeat locus, 3) investigating the effects of and interactions with an important and relevant cardiovascular disease – hypertension (although other pathologies not examined here may also play a role).

6.3. Methods

6.3.1. Sample and procedure

The LBC1936 recruitment, sample and procedure are detailed in Chapter 2 (‘*Methodology’*; Deary et al., 2007; 2012). In addition, at participants cognitive/sociodemographic/medical assessment at the Wellcome Trust Clinical Research Facility (WTCRF) had three standing and sitting blood pressure readings taken, with a B026 Omron 705IT monitor (amongst a wide range of other physical measures which are not examined here; Deary et al., 2007).

6.3.2. Childhood intelligence

The Moray House Test no.12 (MHT) completed at around age 11 years is described in Chapter 2 (*‘Methodology’*). MHT scores were adjusted for age in days at time of assessment, and standardised to an IQ score with a mean of 100 and a standard deviation of 15 for the whole LBC1936 sample.

6.3.3. Genotyping

The genotyping of the *APOE* ε2/ε3/ε4 and *TOMM40* 523 loci is detailed in Chapter 2 (‘*Methodology’*).

6.3.4. Brain MRI: white matter lesions and cerebral microbleeds

The core brain MRI procedure is described in Chapter 2 (‘*Methodology’*). The protocol paper by Wardlaw et al. (2011) details the imaging methodology, briefly described below.

White matter lesion volumes were calculated from binary masks generated by an in-house developed software tool written in MATLAB that applies a technique named Multispectral Colouring Modulation and Variance Identification: 1936 (MCMxxxVI; Valdés

Hernández et al., 2010). This method and measurement is detailed by Wardlaw et al. (2011). Analyses examined white matter lesion volume as a percentage of total intracranial volume (ICV); this is marker of general head size. Analyses also examined lesion volume as a percentage of total brain tissue volume (TBV); this is a marker of current brain size, and therefore takes into account any effects of general age-related brain atrophy on lesion burden (Wardlaw et al., 2011; Valdés Hernández et al., 2010). ICV and TBV were measured as described in Chapter 5 (*'...Hippocampal volume'*), and more specifically the protocol paper by Wardlaw et al. (2011).

Qualitative and quantitative structural imaging assessments are complementary; white matter lesions were scored on the FLAIR and T2W images using the Fazekas scale, which codes separately for deep and periventricular lesions (Fazekas et al., 1987). For each scale, the left and right hemispheres are scored separately. Further details are provided in the protocol paper by Wardlaw et al. (2011).

Microhaemorrhages were coded for number and distribution using a simplified version of the Brain Observer MicroBleed Scale (BOMBS) (Cordonnier et al., 2009), which considers microbleeds as being small, homogenous round foci of low singal intensity on T2*-weighted images of less than 10mm in diameter. This rating scale is used to record the number of observed 'certain' or 'uncertain' microbleeds in the right and left hemispheres, delineated into <5mm and 5-10mm (Cordonnier et al., 2009; see also Wardlaw et al., 2011).

6.4. Statistical analysis

6.4.1. Covariate models & APOE/TOMM40 statistical analysis

The covariate models and analytic strategies for the *APOE* and *TOMM40* gene loci were described in Chapter 2 ('*Methodology'*), and briefly recapped in Chapter 4 ('*…White Matter Integrity*'). All initially-reported *P*-values are raw (and then FDR-adjustment is applied if appropriate), and *P*-values < 0.05 are considered nominally significant.

This chapter differs slightly from the analytic protocol described in Chapter 2 ('*Methodology'*), specifically regarding microbleed analyses. In this chapter, for the microbleed analysis four statistical models were tested to investigate *APOE*-ε and *TOMM40* 523 repeat genetic effects (rather than three, as was done for other imaging/cognitive phenotypes in other chapters). First, all models controlled for gender and age in days at neuroimaging. Significant associations with 'any certain/uncertain cerebral microbleeds' were then re-tested as 'certain' microbleeds only. As described in Chapter 2 (*'Methodology'*), any remaining significant associations were then re-tested additionally controlling for (1) age 11 intelligence, and then (2) the following cardiovascular pathologies; self-reported diagnosis of stroke, high cholesterol, high blood pressure, type-2 diabetes, and any other cardiovascular disease (Schiepers et al., 2012). Analysis of white matter lesions (in terms of covariate models) follows the protocol set in Chapter 2 ('*Methodology'*), recapped in Chapter 4 ('*…White matter integrity'*) and subsequently followed in other chapters.

6.4.2. Hypertension status

Current hypertensive status was defined according to an average of the second and third sitting blood pressure readings around the time of MRI, according to the NeuroCHARGE analysis protocol (a large-scale consortia investigating white matter lesions; Debette et al., 2010; see Appendix A); normotensive (<90 diastolic/140 systolic mmHg with no anti-hypertensive medication; n = 137), hypertensive (>90/140 mmHg, or use of anti-hypertensive medication; n = 598), or severely hypertensive (>100/160 mmHg; n = 122), where individuals on hypertensive medication had 5/10 mmHg added to their respective systolic/diastolic blood pressure scores in calculating hypertensive group. This hypertensive status variable correlated significantly but not perfectly with 'reported history of high blood pressure', both recorded at age 73 (r = 0.38, one-tailed P <0.001, n = 857). The final results reported here reflect the use of the 'hypertensive status' variable, however none of the final results were different when analyses were re-run with the self-reported 'history of high blood pressure' variable unless otherwise specified.

6.4.3. 'BOMBS' microbleed scale

The BOMBS microbleed scale allows a rater to note the number of either uncertain or certain <5mm or 5-10 mm microbleeds in the cortex/grey-white matter junction, subcortical white matter (together 'strictly lobar'), basal ganglia grey matter, internal/external capsules, thalamus (together 'deep'), brainstem and cerebellum (together 'infratentorial') (Cordonnier et al., 2009). To make sure that frequencies would be sufficient for meaningful analysis, new variables were created; 'presence/absence of any microbleeds' (i.e. uncertain + certain), and 'presence/absence of certain microbleeds'. This process was repeated for 'strictly lobar', and 'deep/infratentorial' microbleeds. Table 6.3 shows that presence of 'any uncertain/certain

microbleeds' was generally low. Because of this, the author elected against testing for genetic effects on specific microbleed variables.

6.5. Results

6.5.1. Descriptive statistics

As detailed in Chapter 2 ('*Methodology'*), of the 1091 total LBC1936 participants, 866 attended Waves 1 and 2, and 700 completed neuroimaging at Wave 2. Individuals with MRI data were excluded if they had MMSE scores below 24 or not completed at Wave 2, or reported dementia. Overall, this left 694 participants, of which 659 and 669 participants had successful genotyping for *APOE* ε and *TOMM40* 523, respectively.

APOE had allele frequencies of ε2 = 7.4%, ε3 = 77.0% and ε4 = 15.6%, with genotype frequencies of: ε2/ε2 = 1 (0.2%), ε2/ε3 = 80 (12.1%), ε2/ε4 = 15 (2.3%), ε3/ε3 = 384 (58.3%), ε3/ε4 = 167 (25.3%) and ε4/ε4 = 12 (1.8%). *TOMM40* 523 had allele frequencies of S = 40.9%, L = 15.3% and VL = 43.9%, with genotype frequencies of S/S = 101 (15.1%), S/L = 97 (14.5%), S/VL = 248 (%), L/L = 15 (2.2%), L/VL = 77 (11.5%) and VL/VL = 131 (19.6%). Exact tests confirmed that *APOE* ε and *TOMM40* 523 genotypes were in Hardy-Weinberg equilibrium (*P* values = 0.280 and 0.149 respectively).

Cardiovascular diseases are common risk factors for cerebrovascular pathology and therefore may play a significant role in white matter lesion and microbleed prevalence; the distributions of cardiovascular disease history and hypertensive status (based on blood-pressure taken around the time of MRI) for the final sample are displayed in Table 6.3.

Table 6.3. Descriptive statistics for brain imaging and cardiovascular disease history variables, grouped by apolipoprotein-e *(APOE)* and Translocase of outer mitochondrial membrane 40 (*TOMM40)* genotypes.

	APOE ε genotype					
Imaging/cardiovascular variable	ε2/ε2	ε2/ε3	ε2/ε4	ε3/ε3	ε3/ε4	ε4/ε4
History of diabetes (yes; %)	0 (0)	7 (8.8)	2 (13.3)	45 (11.7)	16 (9.6)	1 (8.3)
History of high cholesterol (yes; %)	1 (100)	24 (30.0)	7 (46.7)	158 (41.1)	80 (47.9)	4 (33.3)
History of Stroke (yes; %)	0 (0)	1 (1.3)	1 (6.7)	33 (8.6)	8 (4.8)	2 (16.7)
History of high blood pressure (yes; %)	1 (100)	34 (42.5)	7 (46.7)	191 (49.7)	87 (52.1)	3 (25.0)
Hypertensive status around MRI (normo-/hyper-/severe-; freq.)	0/1/0	13/58/9	4/11/0	60/270/54	25/118/24	0/2/8/2
History of other cardiovascular pathology (yes; %)	0 (0)	19 (23.8)	3 (20.0)	104 (27.1)	50 (29.9)	4 (33.3)
Presence of uncertain/certain microbleeds (yes; %)	0 (0)	12 (15.4)	0 (0)	37 (10.0)	19 (11.6)	4 (33.3)
Presence of certain microbleeds (yes; %)	0 (0)	8 (10.3)	0 (0)	20 (5.4)	10 (6.1)	2 (16.7)
Fazekas score: Periventricular Left (0/1/2/3; freq.)	0/0/1/0	3/52/20/4	1/11/1/1	11/249/88/25	3/107/49/7	0/11/1/0
Fazekas score: Periventricular Right (0/1/2/3; freq.)	0/0/1/0	4/53/17/5	1/10/2/1	10/254/84/25	5/110/43/8	0/11/1/0
Fazekas score: Periventricular overall (0/1/2/3; freq.)	0/0/1/0	4/50/21/4	1/9/3/1	10/243/95/25	5/104/49/8	0/11/1/0
Fazekas score: Deep, Left (0/1/2/3; freq.)	0/0/1/0	14/47/15/3	5/8/1/0	70/228/69/6	30/102/26/8	1/9/1/1/
Fazekas score: Deep, Right (0/1/2/3; freq.)	0/1/0/0	16/45/17/1	6/7/1/0	71/237/57/8	27/105/26/8	2/9/1/0
Fazekas score: Deep, Overall (0/1/2/3; freq.)	0/1/0/0	13/47/17/2	6/7/1/0	60/237/69/7	24/107/26/9	2/9/1/0
White matter lesion alone in brain tissue volume %	1.98	1.11	0.75	1.04	1.13	0.91
(mean and SD)	(-)	(1.23)	(1.07)	(1.15)	(1.21)	(0.61)
White matter lesion alone in intracranial volume %	1.58	0.87	0.55	0.80	0.87	0.70
(mean and SD)	(-)	(0.96)	(0.79)	(0.89)	(0.93)	(0.47)

	TOMM40 '523' poly-T repeat genotype					
	S/S	S/L	S/VL	L/L	L/VL	VL/VL
History of diabetes (yes; %)	8 (7.9)	12 (12.4)	32 (12.9)	1 (6.7)	4 (5.2)	12 (9.2)
History of high cholesterol (yes; %)	40 (39.6)	49 (50.5)	101 (40.7)	4 (26.7)	31 (40.3)	52 (39.7)
History of Stroke (yes; %)	3 (3.0)	3 (3.1)	24 (9.7)	1 (6.7)	4 (5.2)	9 (6.9)
History of high blood pressure (yes; %)	49 (48.5)	44 (45.4)	116 (46.8)	3 (20.0)	41 (53.2)	73 (55.7)
Hypertensive status around MRI (normo-/hyper-/severe-; freq.)	16/74/11	17/63/17	40/174/34	3/10/2	12/57/8	19/93/19
History of other cardiovascular pathology (yes; %)	26 (25.7)	24 (24.7)	64 (25.8)	5 (33.3)	25 (32.5)	38 (29.0)
Presence of uncertain/certain microbleeds (yes; %)	15 (15.6)	14 (14.7)	22 (9.2)	5 (33.3)	6 (8.0)	13 (10.0)
Presence of certain microbleeds (yes; %)	7 (7.3)	8 (8.4)	15 (6.3)	2 (13.3)	2 (2.7)	9 (6.9)
Fazekas score: Periventricular Left (0/1/2/3; freq.)	5/62/24/6	4/59/29/3	6/160/61/14	0/11/2/2	1/50/21/5	3/90/27/10
Fazekas score: Periventricular Right (0/1/2/3; freq.)	5/63/23/6	5/60/26/4	5/163/58/15	0/11/2/2	2/53/17/5	4/92/24/10
Fazekas score: Periventricular overall (0/1/2/3; freq.)	5/59/27/6	5/57/29/4	5/159/63/14	0/11/2/2	2/48/22/5	4/86/30/10
Fazekas score: Deep, Left (0/1/2/3; freq.)	21/57/17/2	16/60/18/1	40/152/42/7	1/10/2/2	16/44/11/6	24/76/28/2
Fazekas score: Deep, Right (0/1/2/3; freq.)	21/59/15/2	15/60/18/2	42/155/39/5	2/10/2/1	14/46/13/4	27/79/21/3
Fazekas score: Deep, Overall (0/1/2/3; freq.)	18/59/19/1	12/62/19/2	32/161/41/7	2/11/1/1	16/45/11/5	23/75/30/2
White matter lesion alone in brain tissue volume %	1.01	1.14	1.09	1.16	1.12	1.03
(mean and SD)	(1.05)	(1.03)	(1.14)	(1.52)	(1.41)	(1.27)
White matter lesion alone in intracranial volume %	0.78	0.87	0.84	0.90	0.86	0.80
(mean and SD)	(0.81)	(0.77)	(0.88)	(1.18)	(1.10)	(0.99)

Note. S = Short, L = Long, VL = Very long.

6.5.2. White matter lesions

For the *APOE* analyses, no significant effects were found for the ε4 present vs. absent comparison, ε3/ε4 vs. ε3/ε3 comparison or ε2+ vs. ε3/ε3 comparison (see Table 6.4), in terms of white matter lesion mm^3 as a percentage of ICV or TBV, or Fazekas scale scores (periventricular or deep).

For the *TOMM40* 523 analyses, no significant effects were found for brain imaging phenotypes in the whole sample, or when *APOE* ε3/ε4 or ε3/ε3 genotype subgroups were analysed (see Table 6.4).

Table 6.4. *APOE*, *TOMM40* and white matter lesions/cerebral microbleeds.

	APOE ε genotype								
	ε4+ vs. ε4-			ε3/ε4 vs. ε3/ε3			ε2+ vs. ε3/ε3		
Imaging variable	(d.f.) F statistics	*P*	Partial η^2	(d.f.) F statistics	*P*	Partial η^2	(d.f.) F statistics	*P*	Partial η^2
Periventricular Left (Fazekas)	1, 641 = 0.01	0.922	0.000	1, 535 = 0.36	0.551	0.001	1, 449 = 0.08	0.777	0.000
Periventricular Right (Fazekas)	1, 641 = 0.11	0.743	0.000	1, 535 = 0.01	0.939	0.000	1, 449 = 0.46	0.498	0.001
Periventricular overall (Fazekas)	1, 641 = 0.04	0.845	0.000	1, 535 = 0.00	0.974	0.000	1, 449 = 0.42	0.519	0.001
Deep Left (Fazekas)	1, 641 = 0.00	0.951	0.000	1, 535 = 0.20	0.653	0.000	1, 449 = 0.16	0.690	0.000
Deep Right (Fazekas)	1, 641 = 0.30	0.587	0.000	1, 535 = 1.40	0.237	0.003	1, 449 = 0.00	0.987	0.000
Deep Overall (Fazekas)	1, 641 = 0.00	0.956	0.000	1, 535 = 0.57	0.452	0.001	1, 449 = 0.01	0.933	0.000
White matter lesion alone in brain tissue volume (%)	1, 633 = 0.61	0.437	0.001	1, 529 = 1.24	0.266	0.002	1, 445 = 0.02	0.894	0.000
White matter lesion alone in intracranial volume (%)	1, 633 = 0.39	0.534	0.001	1, 529 = 1.04	0.309	0.002	1, 445 = 0.00	0.995	0.000
≥1 uncertain/certain microbleeds	1, 636 = 0.25	0.618	0.000	1, 531 = 0.19	0.592	0.001	1, 446 = 1.35	0.245	0.003
≥1 uncertain/certain lobar microbleeds	1, 636 = 0.01	0.929	0.000	1, 531 = 0.05	0.817	0.000	1, 446 = 0.63	0.429	0.001
≥1 uncertain/certain deep/infratentorial microbleeds	1, 636 = 2.31	0.129	0.004	1, 531 = 1.47	0.225	0.003	1, 446 = 1.14	0.286	0.003

	TOMM40 '523' poly-T repeat genotype								
	Whole sample			*APOE* ε3/ε4 subgroup			*APOE* ε3/ε3 subgroup		
	(d.f.) F statistics	*P*	Partial η^2	(d.f.) F statistics	*P*	Partial η^2	(d.f.) F statistics	*P*	Partial η^2
Periventricular Left (Fazekas)	5, 647 = 0.22	0.952	0.002	1, 159 = 0.01	0.938	0.000	2, 361 = 0.38	0.686	0.002
Periventricular Right(Fazekas)	5, 647 = 0.23	0.951	0.002	1, 159 = 0.38	0.541	0.000	2, 361 = 0.21	0.814	0.001
Periventricular overall(Fazekas)	5, 647 = 0.12	0.987	0.001	1, 159 = 0.08	0.776	0.001	2, 361 = 0.22	0.800	0.001
Deep Left (Fazekas)	5, 647 = 0.63	0.679	0.005	1, 159 = 0.06	0.803	0.000	2, 361 = 1.00	0.369	0.006
Deep Right (Fazekas)	5, 647 = 0.44	0.824	0.003	1, 159 = 0.04	0.839	0.000	2, 361 = 0.23	0.798	0.001
Deep Overall (Fazekas)	5, 647 = 0.35	0.886	0.003	1, 159 = 0.89	0.348	0.006	2, 361 = 0.63	0.535	0.003
White matter lesion alone in brain tissue volume (%)	5, 641 = 0.45	0.812	0.004	1, 156 = 1.83	0.178	0.012	2, 359 = 0.03	0.966	0.000
White matter lesion alone in intracranial volume (%)	5, 641 = 0.37	0.870	0.003	1, 156 = 1.65	0.201	0.010	2, 359 = 0.02	0.978	0.000
≥1 uncertain/certain microbleeds	**5, 642 = 2.77**	**0.018**	**0.021***	1, 157 = 1.32	0.253	0.008	2, 359 = 1.68	0.188	0.009
≥1 uncertain/certain lobar microbleeds	5, 642 = 0.37	0.868	0.003	1, 157 = 1.84	0.177	0.012	2, 359 = 1.05	0.350	0.006
≥1 uncertain/certain deep/infratentorial microbleeds	5, 642 = 0.72	0.606	0.006	1, 157 = 1.24	0.267	0.008	2, 359 = 0.40	0.668	0.002

Note. Age in days at time of testing and gender statistically controlled. Associations significant at $P < 0.05$ are printed in bold-face. *Attenuated to $P > 0.05$ when analysed as 'certain microbleeds' only ($P = 0.478$). Fazekas = Fazekas scale scores.

6.5.3. Cerebral microbleeds

For the *APOE* analyses, no significant effects were found for the ε4 present vs. absent comparison, ε3/ε4 vs. ε3/ε3 comparison or ε2+ vs. ε3/ε3 comparison (see Table 6.4).

For the *TOMM40* 523 analyses, for the most part no significant effects were found. One significant effect was found in the whole sample for 'presence of any uncertain/certain microbleeds' (F [5, 642] = 2.77, P = 0.018, η^2 = 0.021). However, when re-analysed as 'presence of any certain microbleeds', this was not statistically significant (F [5, 642] = 0.90, P = 0.478, η^2 = 0.007). There were no significant effects of *TOMM40* 523 genotype when ε3/ε4 or ε3/ε3 genotype subgroups were analysed (see Table 6.4).

Table 6.5. Hypertensive status (normotensive; hypertensive; severe hypertensive) and white matter lesions/cerebral microbleeds.

Imaging variable	(d.f.) F statistics	*P*	Partial η^2	Post-hoc contrasts (significant at $P < 0.05$)
Periventricular Left (Fazekas)	**2, 674 = 6.30**	**0.002**	**0.018**	Normo<Hyper<Severe
Periventricular Right(Fazekas)	**2, 674 = 5.56**	**0.004**	**0.016**	Normo<Hyper<Severe
Periventricular overall(Fazekas)	**2, 674 = 5.47**	**0.004**	**0.016**	Normo<Hyper; Normo<Severe
Deep Left (Fazekas)	**2, 674 = 7.24**	**0.001**	**0.021**	Normo<Hyper<Severe
Deep Right (Fazekas)	**2, 674 = 5.64**	**0.004**	**0.016**	Normo<Hyper<Severe
Deep Overall (Fazekas)	**2, 674 = 8.91**	**<0.001**	**0.026**	Normo<Hyper<Severe
White matter lesion alone in brain tissue volume (%)	**2, 665 = 6.61**	**0.001**	**0.019**	Normo<Hyper; Normo<Severe
White matter lesion alone in intracranial volume (%)	**2, 665 = 6.54**	**0.002**	**0.019**	Normo<Hyper<Severe
≥1uncertain/certain microbleeds	2, 669 = 2.36	0.095	0.007	
≥1 uncertain/certain lobar microbleed	2, 669 = 0.94	0.390	0.003	
≥1 uncertain/certain deep/infratentorial microbleed	2, 669 = 2.35	0.097	0.007	

Note. Age in days at time of testing and gender statistically controlled. Associations significant at $P < 0.05$ are printed in bold-face. Fazekas = Fazekas scale scores.

Table 6.6. Apolipoprotein-e *(APOE)* translocase of outer mitochondrial membrane 40 (*TOMM40)*, and white matter lesions/cerebral microbleeds: interactions with hypertensive status.

	APOE genotype								
	ε4+ vs. ε4-			ε3/ε4 vs. ε3/ε3			ε2+ vs. ε3/ε3		
Imaging variable	(d.f.) F statistics	*P*	Partial η^2	(d.f.) F statistics	*P*	Partial η^2	(d.f.) F statistics	*P*	Partial η^2
Periventricular Left (Fazekas)	2, 637 = 0.51	0.602	0.002	2, 531 = 0.48	0.687	0.001	2, 445 = 0.93	0.397	0.004
Periventricular Right (Fazekas)	2, 637 = 1.25	0.288	0.004	2, 531 = 1.25	0.288	0.005	2, 445 = 0.14	0.866	0.001
Periventricular overall (Fazekas)	2, 637 = 1.01	0.366	0.003	2, 531 = 0.69	0.501	0.003	2, 445 = 0.29	0.746	0.001
Deep Left (Fazekas)	2, 637 = 0.54	0.584	0.002	2, 531 = 0.32	0.728	0.001	2, 445 = 0.13	0.879	0.001
Deep Right (Fazekas)	2, 637 = 0.29	0.748	0.001	2, 531 = 0.08	0.925	0.000	2, 445 = 0.06	0.945	0.000
Deep, Overall (Fazekas)	2, 647 = 0.97	0.379	0.003	2, 531 = 0.35	0.706	0.001	2, 445 = 0.33	0.718	0.001
White matter lesion alone in brain tissue volume (%)	2, 629 = 0.03	0.971	0.000	2, 525 = 0.27	0.763	0.001	2, 441 = 0.76	0.467	0.003
White matter lesion alone in intracranial volume (%)	2, 629 = 0.04	0.961	0.000	2, 525 = 0.30	0.738	0.001	2, 441 = 0.84	0.432	0.004
≥1 uncertain/certain microbleeds	2, 632 = 0.31	0.734	0.001	2, 527 = 0.02	0.981	0.000	2, 442 = 1.12	0.329	0.005
≥1 uncertain/certain lobar microbleeds	2, 632 = 0.46	0.629	0.001	2, 527 = 0.47	0.625	0.002	2, 442 = 0.79	0.457	0.004
≥1 uncertain/certain deep/infratentorial microbleeds	2, 632 = 1.56	0.209	0.005	2, 527 = 1.03	0.357	0.004	2, 442 = 0.72	0.487	0.003
	TOMM40 '523' poly-T repeat genotype								
	Whole sample			*APOE* ε3/ε4 subgroup			*APOE* ε3/ε3 subgroup		
Periventricular Left (Fazekas)	10, 635 = 1.23	0.271	0.019	2, 155 = 0.64	0.531	0.008	4, 355 = 0.33	0.861	0.004
Periventricular Right (Fazekas)	**10, 635 = 1.96**	**0.036**	**0.030**	2, 155 = 0.94	0.394	0.012	4, 355 = 0.16	0.961	0.002
Periventricular overall (Fazekas)	10, 635 = 1.60	0.104	0.025	2, 155 = 0.69	0.501	0.009	4, 355 = 0.40	0.811	0.004
Deep Left (Fazekas)	10, 635 = 1.16	0.316	0.018	2, 155 = 2.97	0.054	0.037	4, 355 = 0.55	0.700	0.006
Deep Right (Fazekas)	10. 635 = 0.72	0.707	0.011	2, 155 = 1.94	0.147	0.024	4, 355 = 0.36	0.838	0.004
Deep, Overall (Fazekas)	10, 635 = 0.99	0.455	0.015	2, 155 = 1.55	0.216	0.020	4, 355 = 0.90	0.462	0.010
White matter lesion alone in brain tissue volume (%)	10, 629 = 1.19	0.292	0.019	2, 152 = 1.18	0.309	0.015	4, 353 = 0.35	0.846	0.004
White matter lesion alone in intracranial volume (%)	10, 629 = 1.24	0.264	0.019	2, 152 = 1.32	0.269	0.017	4, 353 = 0.36	0.836	0.004
≥1 uncertain/certain microbleeds	10, 630 = 1.34	0.198	0.021	2, 153 = 0.62	0.615	0.542	4, 353 = 2.04	0.089	0.023
≥1 uncertain/certain lobar microbleeds	10, 630 = 1.11	0.352	0.017	2, 153 = 1.09	0.338	0.014	4, 353 = 1.99	0.095	0.022
≥1 uncertain/certain deep/infratentorial microbleeds	10, 630 = 1.41	0.171	0.022	2, 153 = 0.42	0.656	0.005	4, 353 = 1.57	0.182	0.017

Note. Age in days at time of testing and gender statistically controlled. Associations significant at $P < 0.05$ are printed in bold-face. Fazekas = Fazekas scale scores.

6.5.4. Current hypertensive status: main effects and interactions with APOE/TOMM40 genotypes

First analyses tested for a main effect of hypertensive status (normotensive; hypertensive; severely hypertensive, based on readings taken around MRI). Significant effects were found for all white matter lesion variables (percentage white matter lesion in ICV and TBV; periventricular and deep Fazekas scale left/right/overall scores; all $P < 0.01$; see Table 6.5). Post-hoc contrasts showed that in the majority of cases the 'Normotensive' subgroups showed significantly less white matter lesion burden than the 'Hypertensive' subgroup, which in turn showed significantly lower burden than the 'Severe hypertensive' subgroup (See Table 6.5).

A series of interaction models were run (genotype*hypertensive status, controlling for gender and age). There was one statistically significant interaction: where the *TOMM40* 523 genotype interacted with hypertensive status in terms of right periventricular Fazekas scale scores, in the whole sample (F [10, 635] = 1.96, $P = 0.036$, $\eta^2 = 0.030$; see Table 6.6). This was mostly driven by the L/L (mean Fazekas score = 1.04, SD = 0.49, n = 2) and L/VL genotypes (mean = 1.07, SD = 0.26, n = 8) in the 'severe hypertensive' group (all other mean scores ranged = 0.68 – 0.88). Additional analysis was performed where this was re-analysed with 'self-reported history of high blood pressure; yes/no', and this interaction attenuated to non-significance (F [5, 641] = 0.64, $P = 0.673$, $\eta^2 = 0.005$). Because the significant interaction was due to a very small group of individuals, it was not examined further. There was no evidence of any other statistically significant interaction with hypertensive status for either *APOE* ε or *TOMM40* 523 genotypes (all $P > 0.05$; See Table 6.6).

6.6. Discussion

6.6.1. Overview

The current study investigated the effects of the *APOE* ε and *TOMM40* rs10524523 ('523)' poly-T repeat gene loci, on white matter lesion and cerebral microbleed burden as assessed by brain MRI in the LBC1936. This study also investigated interactions with hypertensive status assessed by blood pressure readings taken around the time of MRI, because evidence suggested that this was an important variable, relevant to white matter lesion burden in particular (van Dijk et al., 2004; De Leeuw et al., 2004).

In terms of white matter lesions, this study reports no significant main effects of variation at either gene locus, with lesions analysed either volumetrically - as white matter lesion volume as a percentage of either intracranial volume (i.e. peak brain size), or total brain volume (i.e. current brain size) – or as semi-qualitative Fazekas scale 'periventricular' or 'deep' lesion scores. *TOMM40* 523 genotype significantly interacted with hypertensive status in terms of right periventricular Fazekas scale scores, however there are reasons to doubt this association. First, no other white matter variables showed a significant interaction and it is hard to see why this specific variable would be particularly sensitive to this (vs. right/overall periventricular scores); second, the interaction was not significant when re-analysed as 'self-reported history of high blood pressure', taken around the same time as the MRI (and which would be expected to show relatively similar results as the normo-/hyper-/severely hyper-tensive variable); and finally, the interaction was due to a very small group of individuals in the L/L and L/VL genotypes with severe hypertension. It should also be noted that this is in the context of a large number of tests of association, increasing the risk of type 1 error; generally, it is unlikely that the interaction reflects a genuine mechanism in this sample, and it is not considered any further.

In terms of cerebral microbleeds, *APOE* genotype was not associated with microbleed prevalence, whether analysed overall, strictly lobar or deep/infratentorial. *TOMM40* 523 genotype was associated with significant differences in 'presence of ≥1 uncertain/certain micro bleeds', in the whole sample. However this association attenuated to non-significance when re-analysed as 'certain microbleeds' only; i.e. there was no association when uncertain cerebral microbleeds were excluded, making it unlikely that this effect is genuine.

6.6.2. Interpretation: APOE ε genotype

A recent large scale review by Paternoster et al. concluded that there was no significant association between *APOE* and white matter lesion burden in a pooled analysis of healthy/clinical samples of varying ages. Few of those studies - or those conducted subsequently - examine community dwelling older adults, and therefore may not be directly relevant to non-pathological ageing as investigated here. Examining studies of generally healthy older adults (see Table 6.1) indicates a modest and inconsistent association. The current study, in a large sample of generally healthy older adults, adds significant data to the literature and aligns with Paternoster et al. in observing no significant *APOE*/white matter lesion association. Previous associations may reflect type 1 error (particularly in less reliable, smaller samples), or failure to control for the effects of cardiovascular disease history. Other studies also report broader age ranges that reported here, and this may contribute to the risk of type 1 error; controlling for age is unlikely to eliminate all of the effects of various age related processes that influence white matter lesion burden (Hofer and Sliwinski, 2001).

The current study contrasts with several previous reports in showing no significant association between *APOE* ε4 and cerebral microbleeds. However of those studies two report deleterious effects for the rare ε4/ε4 genotype only (vs. ε3/ε3; Sveinsbjornsdottir et al., 2008; Poels et al., 2011a), or in samples with increased cardiovascular pathology compared with the

present dataset: namely, Vernooij et al. (2008) report slightly increased prevalence of mild hypertension in the Rotterdam Study of older adults (52%) – compared with the current sample (49%; self-reported high blood pressure), and a relatively higher rate of participants having ever smoked (72%, vs. 53% in the final present sample). This prevalence perhaps results in greater differentiation according to genotype, although it is unlikely to account for the whole effect.

A key strength of this study is its use of the BOMBS instrument, because it allows raters to note cerebral microbleeds as either 'certain' or 'uncertain' (examples of mimics include basal ganglia calcification, cortical vessels or partial volume artefacts; Cordonnier et al., 2009). It would be interesting to find if any previously reported significant *APOE*-microbleed associations are affected when analysed to incorporate uncertain microbleeds.

6.6.3. Interpretation: TOMM40 '523' poly-T repeat genotype

The author is not aware of the *TOMM40* 523 locus having being analysed in terms of white matter lesions or cerebral microbleeds in any previous study. Firstly, here this study reports no direct significant association with white matter lesion burden. Previous analyses showed that in this sample - using MRI data taken at the same visit - *TOMM40* 523 genotype was associated with white matter tact integrity assessed with diffusion tensor MRI tractography in the LBC1936, most notably in the left ventral cingulum tract, and a general factor of white matter integrity constructed using principal components analysis (See Chapter 4). The lack of association here highlights that quantitative tractography assesses white matter microstructure rather than gross tissue death, and would therefore be expected to be the more sensitive brain white matter metric. However, the pathology underlying white matter lesions/brain microbleeds may be distinct from that underlying loss of microstructural integrity, meaning that the comparison is not appropriate.

The significant association between *TOMM40* 523 and presence of any uncertain/certain microbleeds attenuated when only certain microbleeds were examined. This indicates that the effect is unlikely to be genuine, or is perhaps the early signs of genuine pathology which may be more clearly apparent in future longitudinal MRI scans conducted in the LBC1936.

6.6.4. Limitations and future research

There is evidence that the prevalence of white matter lesions and microbleeds increase significantly with age (Christiansen et al., 1994). For example, Raz et al. (2012) examined 144 healthy adults of a range of ages (mean age = 58.89, SD = 9.09; range = 44 to 77 years). Participants underwent MRI. Independent of the effects of gender and hypertension, they found a significant effect of age on overall white matter lesion volumes (F [1, 140] = 53.14, *P* <0.001). Poels et al. (2011a) reported that the *APOE* ε4/ε4 allele in 831 older adults (mean age = 68.5, SD = 6.3; vs. ε3/ε3) was associated with an increase of micro bleeds over 3.4 years (range 2.3 to 4.5) (OR = 4.43, 95% C.I's = 1.44 to 13.64). It is possible that the current sample of non-demented, generally healthy older adults (aged around 73 years) have not undergone sufficient change show significant differentiation according to genotype. The sample reported on here (mean age 72.7 years) is undergoing repeat MRI around three years later (i.e. around the year 2012); future studies may investigate *APOE* and *TOMM40* genotypes in terms of longitudinal increase in white matter lesion or cerebral microbleed burden. As discussed above, future studies in independent samples should also use the BOMBS because it allows raters to distinguish between certain and uncertain microbleeds and is therefore likely to be more accurate.

6.6.5. Summary

The current study investigated the effects of variation in the *APOE* ε and *TOMM40* '523' gene loci, in terms of white matter lesion burden and cerebral microbleeds. Previous studies have shown inconsistent and modest associations between *APOE* and these brain imaging phenotypes. In a large study of generally healthy, community dwelling older adults, present analyses ultimately found no association between *APOE* or *TOMM40* and these phenotypes. The present findings align well with recent reviews (Paternoster et al., 2009; Maxwell et al., 2011) that suggest there is unlikely to be a particularly strong or consistent association between *APOE* and these phenotypes in large, well-controlled community dwelling samples. Reasons for discrepancies with previous reports may relate to mean age differences, failure to control for type 1 error e.g. with Bonferroni, and failure to account for cardiovascular disease history. Future studies of *APOE* and cerebral microbleeds should utilise the BOMBS to discriminate between uncertain and certain microbleeds, as was done here.

Chapter 7: *APOE* ε genotype, *TOMM40* poly-T repeat length and cognitive ageing via brain white matter tract integrity

A version of this chapter is in preparation for submission for publication*:* Lyall, D.M., Harris, S.E., Royle, N.A., Bastin, M.E., Valdés Hernández, M. del C., Muñoz Maniega, S., Murray, C., Lutz, M.W., Saunders, A.M., Roses, A.D., Starr, J.M., Porteous, D.J., Wardlaw, J.M. & Deary, I.J.

7.1. Abstract

Specific variations in the *APOE* ε and *TOMM40* '523' poly-T repeat gene loci have been associated with significantly increased risk of Alzheimer's disease (AD). This study investigated the independent effects of these loci on human cognitive ageing, and the extent to which nominally significant gene-cognitive associations were mediated by previously reported genetic associations with white matter tract integrity in this sample. Most participants in the Lothian Birth Cohort 1936 completed a reasoning-type intelligence test at age 11. As part of a longitudinal study of cognitive ageing, they underwent genotyping for *APOE* ε2/ε3/ε4 status and *TOMM40* 523 poly-T repeat length, detailed cognitive/physical assessments and structural brain MRI at a mean age of 72.70 years (standard deviation = 0.74). Data were available from 758-814 subjects for cognitive analysis, and 522-543 for mediation analysis.

APOE genotype was significantly associated with performance on several different tests of cognitive ability, including general factors of intelligence, information processing speed, and memory (raw P values all < 0.05), independent of childhood IQ and cardiovascular disease history. Formal tests of mediation showed that several significant *APOE*-cognitive ageing associations - particularly those related to tests of information processing speed - were significantly, but not entirely mediated by white matter tract integrity. *TOMM40* 523 genotype was significantly associated with scores on two specific tasks, but these associations did not survive statistical correction for *APOE* ε genotype or cardiovascular disease history. A range of brain imaging phenotypes are likely to form the anatomical basis for significant associations between *APOE* genotype and cognitive ageing, including white matter tract microstructural integrity.

7.2. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease characterised by cognitive impairment (Albert et al., 2011). Requisite for the diagnosis of AD is severe cognitive decline that interferes with independent daily life (e.g. the ability to recall and understand information, make reasoned judgements or complete relatively complex tasks; Alzheimer's Association, 2011).

There is evidence from large studies that whereas AD is associated with severe and broad cognitive decline, specific mental domains may be particularly susceptible to the early stages of the dementing process. Chen et al. (2001) were interested in how the degree of specific longitudinal cognitive decline - across two years from 3.5 to 1.5 years prior to diagnosis - predicted the onset of AD. They examined the Monongahela Valley Independent Elders Survey, a large sample of older adults with repeat cognitive testing. Participants (n = 1422) were assessed every two years (over ten years) for the diagnosis of AD. Of those that were diagnosed with AD over the ten years, Chen et al. examined those with complete cognitive data taken around 3.5 and 1.5 years (±1 year) before AD symptoms were first observed. This left 551 older adults overall (mean control group n = 483, age = 72.6, standard deviation [SD] = 4.4; converted-to-AD group n = 68, age = 76.6, SD = 5.3 at baseline). The controls showed no significant declines across two years on any of a broad battery of 15 cognitive tests of different domains, adjusted for age, gender and education. The variables that most strongly predicted conversion to AD were decline in Trail Making Test-B (odds ratio [OR] = 3.72), reflecting processing speed and set-shifting (Lezak et al., 2004) and Word-List delayed recall (OR = 3.63), reflecting verbal memory (Lezak et al., 2004). The decline was not uniform across tests; for example Boston Naming Test (crystal intelligence) performance and Verbal Fluency (semantic memory) performance were not significantly predictive. This study indicates that decline in fluid-type intelligence, processing speed and

memory-related cognitive abilities are particularly sensitive to AD-related pathology (Chen et al., 2001). It is important to understand the mechanisms and neural basis for non-pathological cognitive decline prior to dementia; the primary hypothesis tested in this chapter was that specific aspects of cognitive ability may be particularly susceptible to risk factors for AD.

Two genetic risk factors for AD are in the apolipoprotein-e (*APOE)* and translocase of outer mitochondrial membrane 40 (*TOMM40)* gene loci (Roses et al., 2010). A large number of previous studies have investigated the effects of *APOE* on cognitive ability in later life, including data from the Lothian Birth Cohort 1936 (LBC1936) around age 70, both unadjusted and adjusted for childhood intelligence (i.e. reflecting lifetime cognitive change or 'cognitive ageing'; Deary et al., 2004). Here, this study examined cognitive ability at the older age of around 73 years, and examined whether significant raw associations with *APOE/TOMM40* are mediated by previously reported significant associations between *APOE*/*TOMM40* and white matter microstructural integrity, assessed with diffusion tensor magnetic resonance imaging (DT-MRI; see Chapter 4).

Having described the *APOE* ε and *TOMM40* '523' poly-T repeat gene loci in Chapter 1 ('*Introduction'*) and Chapter 2 ('*Methodology'*), the following sections describe the genes in terms of their relation to cognitive ability in older adults.

7.2.1. APOE ε, TOMM40 523 & cognitive ability

A large number of previous studies have investigated associations between *APOE* genotype and cognitive ability in non-demented adults. Wisdom et al. (2011) conducted a meta-analysis of 77 studies, excluding samples with any disorders that may affect cognitive ability such as dementia or Parkinson's disease (final N = 40,942; calculated mean age = 67.7, SD = 11.86; published between January 2003 and August 2008) that investigated cognitively intact, non-injured individuals assessed with at least one cognitive test, that also provided enough

statistical information to allow for calculation of effect sizes (e.g. means/SD etc). They calculated an effect size, Hedge's g, reflecting the difference between ε4 presence vs. ε4 absence divided by the pooled standard deviation, and therefore corrected for any bias associated with small sample sizes. For analytic purposes, they then constructed a 'd' effect size metric that weighted standardized differences between the groups in terms of sample sizes. They grouped cognitive tests into eight domains – attention, episodic memory, executive function, global ability (e.g. full-scale IQ), perceptual (i.e. information processing) speed, primary (i.e. working) memory, verbal ability (i.e. crystal intelligence) and visuospatial ability. Of a total 227 effect sizes across 8 cognitive domains, significant deleterious effects of the ε4 allele were found for episodic memory (d = -0.14, standardised *r* coefficient = -0.07), global cognitive function (d = -0.05, *r* = 0.03), executive function (d = -0.06, *r* = -0.03) and perceptual speed (d = -0.07, *r* = -0.06; all $P < 0.05$). There were no effects on verbal ability, primary memory, attention or visuospatial functioning (all $P > 0.05$). When Wisdom et al. modelled the effects of age on effect sizes (relative to ε4 presence vs. ε4 absence), they found that increasing sample age significantly predicted greater effect sizes for episodic memory and global cognitive ability only (exact data not provided). These results are similar to those reported by Small et al. (2004), who examined similar studies of *APOE* and non-demented older adults, published from January 1993 to February 2004 (38 independent studies, total N = 20,765). They reported significant deleterious effects of the ε4 allele on episodic memory (d = -0.14, *r* = -0.07, $P < 0.01$), global cognitive ability (d = -0.05, *r* = -0.03, $P < 0.05$), executive functioning (d = -0.06, *r* = -0.03, $P < 0.01$) and perceptual speed (d = -0.07, *r* = -0.04, $P < 0.05$) only. Wisdom et al. note that studies do not consistently correct for important covariates such as gender, education or cardiovascular disease history. Despite this, the high number of papers examined by Wisdom et al. and Small et al. indicate a relatively reliable recent synthesis of the state of the literature.

The largest single report on *APOE* and cognitive ageing involved a large consortium including samples of generally healthy, community-dwelling older adults with a metric of prior cognitive ability (Davies et al., 2012; detailed in Chapter 1; *Introduction*). Davies et al. conducted a genome-wide association study on cognitive ageing based on five large discovery cohorts of community-dwelling, non-demented older adults, totalling 3802 participants. Significant associations were re-tested in two replication cohorts (total N = 1367). The different samples each constructed general factors of cognitive ability with different tests of fluid intelligence, using principal components analysis (PCA). Each score was then adjusted for premorbid ability; either with measures of childhood intelligence or with growth curve models based on data from up to four time points, including ability at 10 years prior. Old age ability adjusted for prior ability was considered 'cognitive ageing'. Participants in the discovery groups received genotyping for *APOE* ε and, in two cohorts, *TOMM40* 523, in addition to GWAS data. When GWAS data for cognitive ageing were meta-analysed, one single nucleotide polymorphism (SNP) achieved genome-wide significance, adjusted for multiple testing (rs2075650 in *TOMM40*, un-standardised $\beta = -1.30$, standard error = 0.27, $P = 2.47 \times 10^{-8}$), with similar results in the smaller replication groups. However the SNP genotyping array used for GWAS (Illumina610-Quadv1 chip; Illumina, San Diego, CA, USA) does not cover the *APOE*/*TOMM40* region well, and the significant association with rs2075650 may reflect linkage with a more causal variant. Davies et al. (2012) therefore tested for the effect of *APOE* ε genotype. They found that possession of the *APOE* ε4 genotype was associated with significantly worse cognitive ageing (un-standardised $\beta = -0.22$, standard error = 0.04, $P = 2.18 \times 10^{-8}$). When rs2075650 was added to the model, it did not reach significance ($P > 0.05$). No significant effects of *TOMM40* 523 genotype were found in pooled meta-analyses.

Luciano et al. (2009a) examined cognitive ageing in the LBC1936 in detail. As described in previous chapters, this sample of older adults completed a test of general intelligence at age 11 – the Moray House Test no. 12 (MHT) – and returned for detailed testing in older age (mean age = 69.9; SD = 0.8; n = 1013). They found a significant deleterious effect of the ε4 allele on Matrix Reasoning (F [1, 1006] = 4.85, *P* = 0.03), Symbol Search (F [1, 1000] = 5.30, *P* = 0.02), Choice Reaction Time (RT) standard deviation (F [1, 992] = 4.64, *P* = 0.03), and a general factor of cognitive ability constructed with PCA (F [1, 981] = 4.24, *P* = 0.04). They did not find any association with on age 70 MHT scores, Verbal Fluency, Letter-Number Sequencing, Backward Digit Span, Block Design, Digit Symbol, Simple RT, or a general factor of Processing Speed. Furthermore, they found that the significant associations survived correction for - and were therefore independent of - age 11 intelligence. In a separate report by Luciano et al. (2009b), the same participants were examined in terms of several immediate and delayed memory tasks at age 70 (n = 1013; Logical Memory; Verbal Paired Associates; Spatial Span). They found a significant deleterious effect of ε4 allele presence for Logical Memory immediate scores (F [1, 983] = 4.32, *P* = 0.04; adjusted or age and gender only), Spatial Span forwards scores additionally adjusted for MHT scores at age 11 years (F [1, 949] = 4.15, *P* = 0.04) and age 70 years (F [1, 983] = 4.50, *P* = 0.03). This indicates significant association independent of early life as well as concurrent general cognitive ability. Luciano et al. (2009a) noted significant main effects of age on all tasks, despite a relatively small age range (P <0.001; except for Simple/Choice RT). This may suggest more pronounced effects of *APOE* genotype now that participants have undergone repeat testing around age 73.

TOMM40 523 genotype and cognitive ability has been examined in three studies (Johnson et al., 2011; Caselli et al., 2012; Hayden et al., 2012). These reports are displayed in Table 7.1 and indicate a generally inconsistent association between *TOMM40* 523 poly-T repeat genotype and cognitive ability.

Table 7.1. Previous studies analysing translocase of outer mitochondrial membrane 40 (*TOMM40*) genotype and cognitive ability in community dwelling older adults.

Report	*Cognitive measures*	*Sample (mean age; SD)*	*n*	*Covariates*	*Genotype effects*	*Notes & limitations*
Johnson et al. 2011	Performance and Verbal IQ, Auditory Verbal Learning Test scores	Healthy older adults (55.4 ±6.00; *APOE* ε3/ε3 genotype only*)	117	Age, intracranial volume, gender (controlled), family history of AD, hypertension (n.s.).	No significant effects of *TOMM40* on performance or verbal IQ; significant deleterious effect of the 523 VL/VL genotype (vs. S/S) on total AVLT scores (49.62 vs. 55.13 out of 75; Cohen's $d = 0.72$, $P < 0.05$)	A sample of individuals with the 'neutral' *APOE* ε3/ε3 genotype
Caselli et al., 2012	Auditory Verbal Learning Test scores	Healthy older adults, longitudinally assessed Every 1-2 years (57.8; ± 12.2*)	639	Age, gender, education (controlled).	Significant difference in memory change between S/S and VL/VL (deleterious) groups (in *APOE ε3/ε3*genotype only: $P = 0.04$).	VL/VL is deleterious in that it shows no test-retest benefit, unlike other genotypes
Hayden et al., 2012	Broad cognitive battery of memory, attention, language and exeutive function (17 tasks)	Healthy older adults (80.6 ± 6.0), then *APOE* ε3ε3 genotype only (n = 82)	127	Age, gender, education, Beck Depression inventory scores (controlled).	No significant effects of 523 in whole sample. Significant deleterious effect of VL allele (Paired Associates Learning and Verbal Recall Memory scores, Rapid Visual Information Processing latency, Digit Span forwards/backwards total; all P <0.05, with S/VL > S/S & VL/VL (*APOE* ε3/ε3 subgroup only)	Deleterious effect of S allele in 'neutral' *APOE* genotype only. S/VL performed much better than S/S and VL/VL, who performed similarly. No associations survived bonferroni correction. No markers of prior ability

* = Weighted estimates.

7.2.2. APOE, TOMM40 and cognitive ageing: mediated by white matter tract integrity?

It is important to understand the anatomical brain substrates of cognitive ageing. Penke et al. (2012) investigated the role of white matter integrity using different metrics, one of which was fractional anisotropy (FA). They reported that a general factor of fractional anisotropy (g_{FA}) constructed with principal components analysis (PCA) was significantly associated with general factors of processing speed (g_{speed}; standardized β = -0.19) and general cognitive ability (*g*; standardized β = 0.13) in LBC1936, explaining around 10% of the variance in general cognitive ability. Few studies have examined the brain imaging phenotypes underpinning the deleterious effects of genetic variation on cognitive ability in old age. In Chapter 2 (‘*ADRB2’*; see also Lyall et al., 2013), mediation analysis was conducted in the LBC1936, where a gene-cognitive ageing association was significantly mediated by integrity in a specific white matter tract. Specifically, variation in the rs1042714 SNP (in the *ADRB2* gene) was associated with performance on the Digit Symbol Coding test (standardised β = -0.09, *P* = 0.010), and integrity of the left arcuate fasciculus tract (assessed by FA; standardised β = -0.11, *P* = 0.007). It was reported that the SNP-cognitive ageing association was mediated by left arcuate fasciculus FA (bootstrapping point estimate coefficient = -0.207, 95% C.I’s = -0.52 to -0.04; Lyall et al. 2013).

This PhD thesis has tested for genetic associations with white matter microstructural integrity, hippocampal volumes (independent of generalized brain atrophy and head size), brain white matter lesions and cerebral microbleeds. Those studies showed significant nominal raw effects of *APOE* ε and *TOMM40* 523 genotypes on white matter microstructure only, and lower integrity in these specific associated tracts may have effects on cognitive ability.

7.2.3. The current study

The *APOE* ε and *TOMM40* 523 poly-T repeat gene loci have been associated with increased risk of AD. Previous studies have investigated the effects of *APOE* on cognitive ability in older adults; however, there are relatively few studies that examine *TOMM40* 523 genotype, and the author is not aware of any studies that have formally investigated the specific structural brain MRI phenotypes that mediate *APOE*-cognitive associations.

This study aims to: 1) assess the effects of *APOE/TOMM40* 523 genotypes on cognitive abilities in older people, at first unadjusted and then adjusted for age 11 intelligence (i.e. reflecting cognitive ageing); and 2) determine the extent to which any such significant associations are mediated by previously-reported associations with white matter tract integrity, assessed with DT-MRI (see Chapter 4).

7.3. Methods

7.3.1. Sample and procedure

The LBC1936 recruitment, sample and procedure are detailed in Chapter 2 (‘*Methodology’*; Deary et al., 2007; 2012).

7.3.2. Genotyping

The genotyping of the *APOE* ε2/ε3/ε4 and *TOMM40* 523 loci is detailed in Chapter 2 (‘*Methodology’*).

7.3.3. Childhood intelligence

The Moray House Test no.12 (MHT) completed at around age 11 years is described in Chapter 2 (*'Methodology'*). MHT scores were adjusted for age in days at time of assessment, and standardised to an IQ score with a mean of 100 and a standard deviation of 15 for the whole LBC1936 sample.

7.3.4. Assessment of cognitive domains at age ~73 ('Wave 2')

The tests are described in Chapter 2 (*'Methodology'*; Deary et al., 2007). In brief, the cognitive domains assessed were as follows. Working memory was assessed with Digit Span Backwards and Letter Number Sequencing from the WAIS-III^{UK} (Wechsler 1998a). Processing speed was assessed using: Digit Symbol Coding and Symbol Search from the WAIS-III^{UK}; Simple reaction time (RT), and Four-Choice RT via a self-contained device (Cox et al. 1993); and a psychophysical visual discrimination task called Inspection Time (Deary et al. 2007). Verbal declarative memory was assessed with the Verbal Paired Associates I and II, and Logical Memory I and II tests from the Wechsler Memory Scale-III III^{UK} (WMS-III^{UK}; Wechsler 1998b). Visuospatial ability and memory were assessed with Block Design and Spatial Span from the WAIS-III^{UK} and WMS-III^{UK} respectively. Abstract reasoning was assessed using Matrix Reasoning from the WAIS-III^{UK}.

Data reduction was applied to the test scores using PCA, as described in Chapter 2 (*'Methodology'*). The first unrotated principal component score in each domain for general intelligence (*g*), information processing speed (g_{speed}) and memory (g_{memory}) accounted for 50-54% of the respective variance, and all individual variables had high loadings on their respective first unrotated components.

7.3.5. Diffusion MRI and tractography

The core brain MRI procedure is described in Chapter 2 ('*Methodology'*), and the diffusion-tensor procedure and processing is described in Chapter 3 ('*ADRB2…*'; but see Wardlaw et al., 2011 for specific details). To permit principal components analysis (PCA) on the tractography data, participants with up to two missing values from specific tracts had data replaced with the mean value for that tract. PCA was conducted on tract-averaged water diffusion parameters for twelve pathways, giving a clear single-factor model for FA (g_{fa}) that accounted for 38.8% of the overall variance. The ventral cingulum was not included in the PCA because the rostral and ventral cingula are subdivisions of the same tract. General white matter integrity factors have previously been found to be associated with cognitive abilities in this sample (Penke et al., 2012; Penke et al., 2010a).

7.4. Statistical Analysis

7.4.1. Covariate models & APOE/TOMM40 statistical analysis

The covariate models and analytic strategies for the *APOE* and *TOMM40* gene loci were described in Chapter 2 ('*Methodology'*). For ease, this is recapped in Table 7.2 also. All initially-reported *P*-values are raw (and then FDR-adjustment is applied), and *P*-values < 0.05 are considered nominally significant.

Table 7.2. Final analytic strategies for *APOE* ε and *TOMM40* '523' poly-T repeat gene loci for Chapters 4-7.

	APOE ε	*TOMM40 '523'*
Gene locus:	rs7412 + rs429358 (simply 'ε genotype')	Poly-T repeat at rs10524523
Step 1:	ε4 present (vs. ε4 absent)	Overall effect of genotype? (S/S; S/L; VL/L; L/L; VL/VL) (in the whole sample)
Step 2:	ε3/ε4 (vs. ε3/ε3)	S/S; S/L*; L*/L* (in *APOE* ε3/ε4 genotype subgroup)
Step 3:	ε2 present (vs. ε3/ε3)	S/S; S/L*; L*/L* (in *APOE* ε3/ε3 genotype subgroup)
Covariate models:	Model 1: Age + Gender Model 2: Age + Gender + Age 11 IQ Model 3: Age + Gender + Age 11 IQ + Cardiovascular disease history	

Note. The term 'ε4 present' includes participants with ε3/ε4; ε2/ε4; ε4/ε4 genotypes pooled together, while 'ε4 absent' includes all other genotypes. 'ε2' present includes participants with ε2/ε3 and ε3/ε3 genotypes only. *TOMM40* 523 L* = L and VL alleles pooled.

7.4.2. Mediation analysis

Mediation analysis was used to test the indirect effect of the predictor variable (*APOE/TOMM40*) upon the outcome (cognitive function), through the hypothesised mediator (white matter integrity). Mediation analysis was run using the INDIRECT bootstrapping macro (Preacher and Hayes, 2008), and detailed in Chapter 3 '(*ADRB2…*'). Briefly, in a simple example mediation model, variable X's effects on variable Y can be either direct or

indirect via variable M. Path A represents the effect of X on M, while path B represents the effect of M on Y, partialling out the effect of X. The direct effect of X on Y is represented by path C. The indirect effect can then be quantified as the combined product of paths A and B. The bias-corrected bootstrapping point estimate coefficients that are reported here each reflect this indirect product (Preacher and Hayes, 2008). Bootstrapping point estimate coefficients are un-standardised and averaged over 5000 bootstrap estimates (Preacher and Hayes, 2008). The indirect point estimate coefficients (commonly simply 'effects') were considered statistically significant if the 95% confidence intervals (C.I's) did not cross 0.00 (Preacher & Hayes, 2008)

7.5. Results

7.5.1. Descriptive statistics

As detailed in Chapter 2 ('*Methodology'*), of the 1091 total LBC1936 participants, 866 attended Waves 1 and 2. Individuals who had MMSE scores below 24, did not complete the MMSE at Wave 2 or had a history of dementia were excluded from analysis. Overall, this left 859 participants, of which 811 and 823 participants had successful genotyping for *APOE* and *TOMM40*, respectively.

APOE had allele frequencies of ε2 = 7.3%, ε3 = 76.9% and ε4 = 15.8%, with genotype frequencies of: ε2/ε2 = 3 (0.4%), ε2/ε3 = 95 (11.7%), ε2/ε4 = 18 (2.2%), ε3/ε3 = 472 (58.2%), ε3/ε4 = 208 (25.6%), and ε4/ε4 = 15 (1.8%) (total n = 811). *TOMM40* 523 had allele frequencies of S = 41.0%, L = 15.4% and VL = 43.6%, with genotype frequencies of S/S = 125 (15.2%), S/L = 123 (14.9%), S/VL = 302 (36.7%), L/L = 18 (2.2%), L/VL = 95 (11.5%) and VL/VL = 160 (19.4%) (total n = 823). Exact tests confirmed that *APOE* and

TOMM40 were in Hardy-Weinberg equilibrium (*P*-values = 0.44 and 0.06, respectively). Specific GLM data (F/*P* value/η^2) are presented in Tables 7.3 to 7.8.

APOE, TOMM40 and cognitive ability - not adjusted for childhood intelligence (Model 1)

Significant deleterious effects of the *APOE* ε4 allele (vs. absence) were found on three tasks: specifically Symbol Search, Inspection Time Total, and Spatial Span ('*Step 1'*). For the ε3/ε3 vs. ε3/ε4 comparison a significant effect was found for Inspection Time Total only ('*Step 2'*). For the ε2+ (vs. ε3/ε3) comparison, a significant protective effect of ε2 possession was found for Inspection Time Total (all $P < 0.05$; '*Step 3*'; see Table 7.3).

For *TOMM40*, no significant effects were found in the whole sample ('*Step 1'*) or ε3/ε3 genotype subgroup ('*Step 3*'). A single significant protective effect of the S allele was found in *APOE* ε3/ε4 genotype subgroup only, for Letter-Number Sequencing ($P < 0.05$; '*Step 2'*; see Table 7.4).

Table 7.3. *APOE ε* and cognitive ability at age 73 - not adjusted for age 11 IQ.

Cognitive test	ε4 allele presence (vs. absence)			ε3/ε4 (vs. ε3/ε3)			*APOE* ε2/ε3 & ε2/ε2 (vs. ε3/ε3)		
	(d.f.) F statistics	*P*	Partial η^2	(d.f.) F statistics	*P*	Partial η^2	(d.f.) F statistics	*P*	Partial η^2
Age 11 IQ	(1, 757) = 0.75	0.386	0.001	(1, 633) = 0.52	0.473	0.001	(1, 529) = 1.73	0.189	0.003
General factor: intelligence (g)	(1, 797) = 0.19	0.166	0.002	(1, 667) = 1.58	0.210	0.002	(1, 559) = 0.66	0.417	0.001
Digit Span Backwards	(1, 807) = 0.03	0.862	0.000	(1, 676) = 0.21	0.649	0.000	(1, 566) = 2.92	0.088	0.005
Matrix Reasoning	(1, 806) = 1.40	0.237	0.002	(1, 675) = 1.84	0.175	0.003	(1, 565) = 0.08	0.782	0.000
Block Design	(1, 805) = 1.77	0.184	0.002	(1, 674) = 1.96	0.162	0.003	(1, 564) = 1.84	0.176	0.003
Letter-Number Sequencing	(1, 806) = 0.10	0.747	0.000	(1, 675) = 0.00	0.954	0.000	(1, 565) = 0.57	0.449	0.001
General factor: processing speed (g_{Speed})	(1, 765) = 2.53	0.112	0.003	(1, 639) = 0.62	0.431	0.001	(1, 543) = 1.51	0.220	0.003
Digit Symbol Coding	(1, 804) = 0.67	0.414	0.001	(1, 673) = 0.02	0.896	0.000	(1, 564) = 1.08	0.298	0.002
Symbol Search	**(1, 802) = 3.92**	**0.048**	**0.005**	(1, 671) = 0.20	0.153	0.003	(1, 562) = 0.00	0.960	0.000
Simple Reaction time (seconds)	(1, 798) = 0.01	0.914	0.000	(1, 668) = 0.50	0.478	0.001	(1, 559) = 0.26	0.107	0.005
Four Choice Reaction Time (seconds)	(1, 802) = 0.10	0.748	0.000	(1, 672) = 0.20	0.652	0.000	(1, 564) = 0.15	0.230	0.003
Inspection Time Total	**(1, 799) = 8.42**	**0.004**	**0.011**	**(1, 651) = 3.96**	**0.047**	**0.006**	**(1, 552) = 0.59**	**0.015**	**0.011**
General factor: memory (g_{Memory})	(1, 788) = 1.94	0.165	0.002	(1, 659) = 1.90	0.169	0.003	(1, 552) = 0.04	0.848	0.000
Logical Memory	(1, 805) = 0.32	0.075	0.004	(1, 674) = 2.48	0.116	0.004	(1, 565) = 0.41	0.523	0.001
Verbal Paired Associates	(1, 790) = 0.27	0.603	0.000	(1, 661) = 0.32	0.570	0.000	(1, 553) = 0.25	0.615	0.000
Spatial Span	**(1, 803) = 0.46**	**0.033**	**0.006**	(1, 672) = 3.50	0.062	0.005	(1, 562) = 0.09	0.762	0.000

Note. Age in days at time of testing and gender statistically controlled. Associations significant at $P < 0.05$ are printed in bold-face and italics.

Table 7.4. *TOMM40 '523'* poly-T repeat length genotype and cognitive ability - not adjusted for age 11 IQ.

	Step 1 Whole sample			*Step 2* ε3/ε4 carriers only			*Step 2* ε3/ε3 carriers only		
Cognitive test	(d.f.) F statistics	*P*	Partial η^2	(d.f.) F statistics	*P*	Partial η^2	(d.f.) F statistics	*P*	Partial η^2
Age 11 IQ	5, 763 = 0.31	0.908	0.002	(1, 188) = 0.00	0.985	0.000	(2, 427) = 0.88	0.417	0.004
General factor: intelligence (g)	5, 805 = 0.49	0.784	0.003	(1, 198) = 0.70	0.405	0.004	(2, 449) = 0.72	0.489	0.003
Digit Span Backwards	5, 815 = 0.10	0.992	0.001	(1, 200) = 0.07	0.788	0.000	(2, 458) = 0.53	0.591	0.002
Matrix Reasoning	5, 814 = 0.59	0.707	0.004	(1, 200) = 1.65	0.201	0.008	(2, 457) = 0.43	0.654	0.002
Block Design	5, 813 = 0.74	0.597	0.005	(1, 200) = 0.35	0.556	0.002	(2, 455) = 1.20	0.301	0.005
Letter-Number Sequencing	5, 814 = 1.07	0.378	0.007	**(1, 200) = 4.48**	**0.035**	**0.022**	(2, 457) = 1.28	0.278	0.006
General factor: processing speed (g_{Speed})	5, 773 = 0.42	0.839	0.003	(1, 185) = 0.16	0.691	0.001	(2, 437) = 0.06	0.944	0.000
Digit Symbol Coding	5, 812 = 0.73	0.603	0.004	(1, 199) = 0.32	0.570	0.002	(2, 456) = 0.99	0.371	0.004
Symbol Search	5, 810 = 0.83	0.526	0.005	(1, 199) = 0.01	0.916	0.000	(2, 454) = 0.59	0.554	0.003
Simple Reaction time (seconds)	5, 806 = 0.45	0.816	0.003	(1, 198) = 0.28	0.596	0.001	(2, 452) = 0.97	0.382	0.004
Four Choice Reaction Time (seconds)	5, 810 = 0.38	0.860	0.002	(1, 198) = 0.95	0.332	0.005	(2, 456) = 0.25	0.783	0.001
Inspection Time Total	5, 787 = 1.79	0.113	0.011	(1, 189) = 1.57	0.211	0.008	(2, 445) = 0.36	0.700	0.002
General factor: memory (g_{Memory})	5, 796 = 1.27	0.276	0.008	(1, 195) = 0.16	0.691	0.001	(2, 446) = 0.43	0.650	0.002
Logical Memory	5, 813 = 1.50	0.189	0.009	(1, 199) = 0.09	0.767	0.000	(2, 457) = 0.53	0.587	0.002
Verbal Paired Associates	5, 798 = 0.68	0.640	0.004	(1, 196) = 0.02	0.899	0.000	(2, 447) = 1.01	0.365	0.004
Spatial Span	5, 811 = 2.21	0.051	0.004	(1, 200) = 3.48	0.064	0.017	(2, 454) = 0.85	0.428	0.004

Note. Age in days at time of testing and gender statistically controlled. Associations significant at $P < 0.05$ are printed in bold-face.

7.5.2. APOE, TOMM40 and cognitive ability - adjusted for childhood intelligence (Models 2 & 3)

Significant deleterious effects of *APOE* ε4 allele presence (vs. absence) were found for nine out of a possible fifteen age 73 cognitive ageing variables (see Table 7.5). Specifically these were *g*, Matrix Reasoning, $g_{speed,}$ Digit Symbol Coding, Symbol Search, Inspection Time Total, g_{memory}, Logical Memory total, and Spatial Span (all $P < 0.05$; *'Step 1'*; Table 7.5) Each significant association survived correction for cardiovascular disease history ($P < 0.05$; see Table 7.7).

For the ε3/ε4 vs. ε3/ε3 comparison, significant deleterious effects of ε3/ε4 were found for: *g*, Matrix Reasoning, Block Design, Symbol Search, Inspection Time Total, g_{memory}, Logical Memory total and Spatial Span (all $P < 0.05$; see Table 7.5; i.e. similar to above except for Digit Symbol Coding and g_{speed} which were not significant here, and Block Design which was significant here). All associations survived correction for cardiovascular disease history (All $P < 0.05$; '*Step 2'*; see Table 7.7) except for Symbol Search ($P > 0.05$).

For the ε2+ (vs. ε3/ε3) comparison, a significant protective effect of ε2 allele possession was found for Inspection Time Total ($P < 0.05$; Table 7.5). This survived correction for cardiovascular disease history ($P < 0.05$; '*Step 3'*; see Table 7.7).

For *TOMM40* 523, two significant protective associations with S allele possession were found: in the whole sample for Spatial Span ('*Step 1'*), and for Letter-Number Sequencing in the *APOE* ε3/ε4 genotype subgroup only (both $P < 0.05$; '*Step 2'*; see Table 7.6). The significant association with Spatial Span scores did not remain significant when corrected for possession of the *APOE* ε4 allele ($F [5, 730] = 1.62$, $P = 0.151$, $\eta^2 = 0.011$). The significant association with Letter-Number Sequencing attenuated to (marginal) non-significance when corrected for cardiovascular disease history ($P = 0.050$, rounded down; see

Table 7.7). Because of this, associations between *TOMM40* and cognitive ageing were not examined further.

7.5.3. Correction for multiple testing with False Discovery Rate

When tests of main effect were corrected for multiple testing with the FDR, all significant associations attenuated to non-significance (all FDR-adjusted *P* values > 0.05). Further exploratory analyses were conducted on the basis that they could provide directions for future studies.

Table 7.5. *APOE ε* and cognitive ability at age 73, adjusted for age 11 IQ.

	ε4 allele presence (vs. absence)			ε3/ε4 (vs. ε3/ε3)			*APOE* ε2/ε3 & ε2/ε2 (vs. ε3/ε3)		
Cognitive test	(d.f.) F statistics	*P*	Partial η^2	(d.f.) F statistics	*P*	Partial η^2	(d.f.) F statistics	*P*	Partial η^2
General factor: intelligence (g)	**(1, 746) = 8.04**	**0.005**	**0.011**	**(1, 622) = 7.32**	**0.007**	**0.012**	(1, 519) = 0.08	0.773	0.000
Digit Span Backwards	(1, 755) = 0.53	0.468	0.001	(1, 631) = 1.12	0.291	0.002	(1, 527) = 2.52	0.113	0.005
Matrix Reasoning	**(1, 754) = 4.19**	**0.041**	**0.006**	**(1, 630) = 5.54**	**0.019**	**0.009**	(1, 526) = 0.02	0.890	0.000
Block Design	(1, 753) = 3.70	0.055	0.005	**(1, 629) = 4.68**	**0.031**	**0.007**	(1, 525) = 1.91	0.168	0.004
Letter-Number Sequencing	(1, 754) = 0.70	0.403	0.001	(1, 630) = 0.43	0.513	0.001	(1, 526) = 0.66	0.418	0.001
General factor: processing speed (g_{Speed})	**(1, 716) = 5.80**	**0.016**	**0.008**	(1, 597) = 2.44	0.119	0.004	(1, 506) = 2.06	0.152	0.004
Digit Symbol Coding	**(1, 753) = 3.92**	**0.048**	**0.005**	(1, 629) = 1.61	0.205	0.003	(1, 525) = 1.44	0.231	0.003
Symbol Search	**(1, 750) = 6.04**	**0.014**	**0.008**	**(1, 626) = 3.86**	**0.050**	**0.006**	(1, 523) = 0.04	0.849	0.000
Simple Reaction time (seconds)	(1, 747) = 0.04	0.845	0.000	(1, 624) = 0.22	0.638	0.000	(1, 521) = 3.69	0.055	0.007
Four Choice Reaction Time (seconds)	(1, 751) = 0.30	0.587	0.000	(1, 628) = 0.03	0.866	0.000	(1, 525) = 1.47	0.226	0.003
Inspection Time Total	**(1, 729) = 11.10**	**0.001**	**0.015**	**(1, 608) = 6.34**	**0.012**	**0.010**	**(1, 514) = 5.96**	**0.015**	**0.011**
General factor: memory (g_{Memory})	**(1, 739) = 5.43**	**0.020**	**0.007**	**(1, 617) = 5.61**	**0.018**	**0.009**	(1, 515) = 0.38	0.536	0.001
Logical Memory	**(1, 753) = 6.23**	**0.013**	**0.008**	**(1, 629) = 5.10**	**0.024**	**0.008**	(1, 526) = 1.20	0.274	0.002
Verbal Paired Associates	(1, 741 = 1.03	0.311	0.001	(1, 619) = 1.36	0.245	0.002	(1, 516) = 0.45	0.501	0.001
Spatial Span	**(1, 751) = 7.10**	**0.008**	**0.009**	**(1, 627) = 6.30**	**0.012**	**0.010**	(1, 523) = 0.02	0.883	0.000

Note. Age in days at time of testing, gender and age 11 IQ statistically controlled. Associations significant at $P < 0.05$ are printed in bold-face.

Table 7.6. *TOMM40 '523'* poly-T repeat length genotype and cognitive ability - adjusted for age 11 IQ.

	Step 1 Whole sample			*Step 2* ε3/ε4 carriers only			*Step 2* ε3/ε3 carriers only		
Cognitive test	(d.f.) F statistics	*P*	Partial η^2	(d.f.) F statistics	*P*	Partial η^2	(d.f.) F statistics	*P*	Partial η^2
General factor: intelligence (g)	5, 753 = 1.52	0.182	0.010	(1, 186) = 1.04	0.309	0.006	(2, 418) = 0.92	0.401	0.004
Digit Span Backwards	5, 762 = 0.08	0.995	0.001	(1, 187) = 0.07	0.786	0.000	(2, 426) = 0.74	0.476	0.003
Matrix Reasoning	5, 761 = 0.73	0.602	0.005	(1, 187) = 1.02	0.314	0.005	(2, 425) = 0.56	0.572	0.003
Block Design	5, 760 = 1.39	0.228	0.009	(1, 187) = 0.25	0.618	0.001	(2, 424) = 1.31	0.277	0.006
Letter-Number Sequencing	5, 761 = 1.19	0.312	0.008	**(1, 187) = 4.51**	**0.035**	**0.024**	(2, 425) = 1.39	0.250	0.007
General factor: processing speed (g_{Speed})	5, 723 = 0.93	0.463	0.006	(1, 173) = 0.31	0.578	0.002	(2, 407) = 0.30	0.739	0.001
Digit Symbol Coding	5, 760 = 0.93	0.459	0.006	(1, 187) = 0.31	0.578	0.002	(2, 424) = 0.89	0.410	0.004
Symbol Search	5, 757 = 1.18	0.316	0.008	(1, 186) = 0.00	0.951	0.000	(2, 422) = 0.37	0.692	0.002
Simple Reaction time (seconds)	5, 754 = 0.55	0.741	0.004	(1, 185) = 0.42	0.518	0.002	(2, 421) = 1.25	0.287	0.006
Four Choice Reaction Time (seconds)	5, 758 = 0.35	0.880	0.002	(1, 186) = 0.84	0.462	0.004	(2, 424) = 0.44	0.646	0.002
Inspection Time Total	5, 736 = 2.13	0.060	0.014	(1, 177) = 2.26	0.135	0.013	(2, 414) = 0.20	0.820	0.001
General factor: memory (g_{Memory})	5, 746 = 2.11	0.063	0.014	(1, 183) = 0.17	0.685	0.001	(2, 416) = 0.79	0.457	0.004
Logical Memory	5, 760 = 2.13	0.060	0.014	(1, 186) = 0.27	0.601	0.001	(2, 425) = 2.33	0.099	0.011
Verbal Paired Associates	5, 748 = 0.95	0.449	0.006	(1, 184) = 0.02	0.891	0.000	(2, 417) = 1.09	0.337	0.005
Spatial Span	**5, 758 = 2.31**	**0.043**	**0.015**	(1, 187) = 3.09	0.081	0.016	(2, 422) = 1.25	0.288	0.006

Note. Age in days at time of testing, gender and age 11 IQ statistically controlled. Associations significant at $P < 0.05$ are printed in bold-face.

Table 7.7. Significant associations between *APOE* & *TOMM40* and cognitive ageing in Tables 7.4 and 7.5 – adjusted for cardiovascular disease history (in addition to age, gender, and age 11 IQ)

	APOE ε genotype								
	ε4+ vs. ε4-			ε3/ε4 vs. ε3/ε3			ε2+ vs. ε3/ε3		
Brain imaging variable	(d.f.) F statistics	*P*	Partial η^2	(d.f.) F statistics	*P*	Partial η^2	(d.f.) F statistics	*P*	Partial η^2
General factor: intelligence (g)	1, 741 = 8.47	0.004	0.011	1, 617 = 7.78	0.005	0.012	-		
Matrix Reasoning	1, 749 = 4.41	0.036	0.006	1, 625 = 5.74	0.017	0.009	-		
Block Design	-			1, 624 = 5.36	0.021	0.009	-		
General factor: processing speed (g_{Speed})	1, 711 = 6.36	0.012	0.009	-			-		
Digit Symbol Coding	1, 748 = 4.27	0.039	0.006	-			-		
Symbol Search	1, 745 = 5.72	0.017	0.008	1, 621 = 3.56	0.060	0.006	-		
Inspection Time Total	1, 724 = 11.36	0.001	0.015	1, 603 = 6.58	0.011	0.011	1, 509 = 4.93	0.027	0.010
General factor: memory (g_{Memory})	1, 745 = 5.36	0.021	0.007	1, 612 = 5.41	0.020	0.009	-		
Logical Memory	1, 748 = 6.22	0.013	0.008	1, 624 = 5.02	0.025	0.008	-		
Spatial Span	1, 746 = 6.03	0.014	0.008	1, 622 = 5.31	0.022	0.008	-		
	TOMM40 '523' genotype								
	Whole sample			*APOE* ε3/ε4 subgroup			*APOE* ε3/ε3 subgroup		
	(d.f.) F statistics	*P*	Partial η^2	(d.f.) F statistics	*P*	Partial η^2	(d.f.) F statistics	*P*	Partial η^2
Letter Number Sequencing	-			1, 182 = 3.88	0.050	0.021	-		

Table 7.8. Inter-correlations between cognitive ageing and white matter tract variables that are each significantly associated with *APOE* ε genotype.

r (*P* value)	Right ventral cingulum FA	Left inferior longitudinal fasciculus FA
General factor: intelligence (*g*)	0.02 (0.347)	**0.20 (<0.001)**
Matrix Reasoning	-0.01 (0.411)	**0.10 (0.008)**
Block Design	0.06 (0.077)	**0.16 (<0.001)**
General factor: processing speed (g_{Speed})	0.04 (0.196)	**0.17 (<0.001)**
Digit Symbol Coding	0.03 (0.276)	**0.18 (<0.001)**
Symbol Search	-0.03 (0.240)	**0.11 (0.004)**
Inspection Time Total	**0.07 (0.042)**	**0.14 (<0.001)**
General factor: memory (g_{Memory})	0.01 (0.441)	**0.08 (0.024)**
Logical Memory total	0.02 (0.328)	0.05 (0.131)
Spatial Span	-0.02 (0.334)	**0.09 (0.015)**

Note. Pearson semi-partial correlations controlling for age, gender and age 11 IQ. Figures reflect '*r*' correlations. FA = fractional anisotropy. Significant correlations in bold reflect a significant correlation ($P < 0.05$) where both variables are also associated with *APOE* genotype (see Table 7.7).

Mediation: inter-correlations between white matter tract integrity & cognitive ageing

Analyses next examined the mediation of genetic-cognitive ageing associations, via white matter tract integrity metrics. First I examined inter-correlations between white matter tract variables which showed significant association with *APOE* genotype in Chapter 4, and cognitive ageing variables which were significantly associated with *APOE* here after correction for cardiovascular disease history (see Tables 7.7 & 7.8). No significant associations were found between *TOMM40* and cognitive ageing, corrected for cardiovascular disease history or *APOE* genotype. As can be seen in Table 7.8, several semi-partial correlations between white matter tract FA and cognitive ability were statistically significant, controlling for age, gender and age 11 IQ, where both variables were also associated with *APOE* genotype (detailed below).

There were significant correlations between variables that were associated with *APOE*: for right ventral cingulum FA with Inspection Time Total (r = 0.07, P = 0.042), and for left inferior longitudinal fasiculus with g (r = 0.20, P < 0.001), Matrix Reasoning (r = 0.10, P = 0.008), Block Design (r = 0.16, P < 0.001), g_{speed} (r = 0.17, P <0.001), Digit Symbol Coding (r = 0.18, P < 0.001), Symbol Search (r = 0.11, P = 0.004), Inspection Time Total, r = 0.14, P <0.001), g_{memory} (r = 0.08, P = 0.024) and Spatial Span (r = 0.09, P = 0.015).

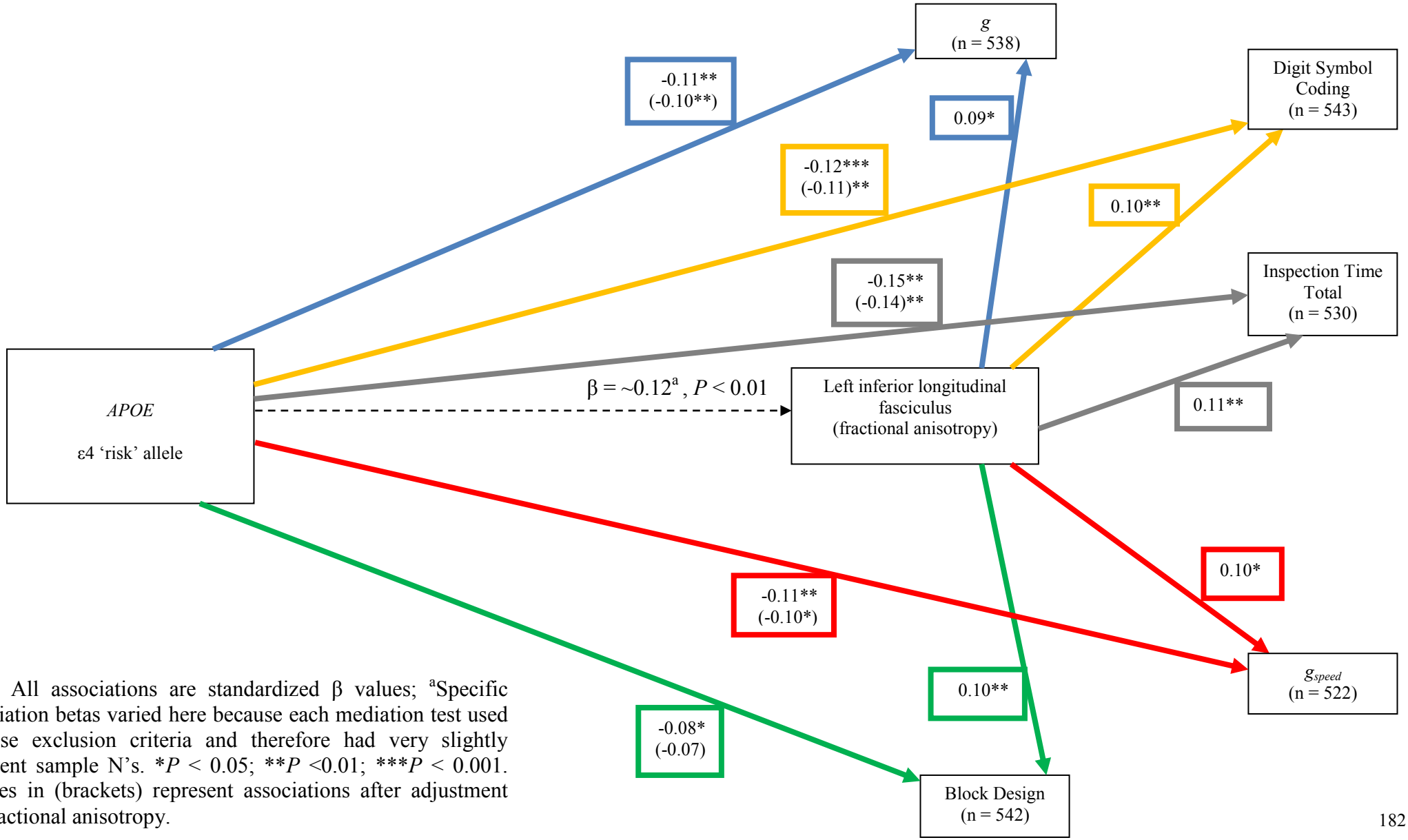
7.5.4. Mediation: APOE/TOMM40 → white matter tract integrity → cognitive ageing

Brain imaging and cognitive variables that showed nominally significant associations with *APOE* genotypes, and that significantly inter-correlated, were examined further for mediation. Analyses tested exploratory mediation based on the above correlations, with the bootstrapping technique (Preacher and Hayes, 2008).

Bootstrapping statistics indicated that right ventral cingulum FA did not significantly mediate the association between *APOE* and Inspection Time Total scores (indirect effect = -0.16, 95% C.I's = -0.57 to 0.02).

Bootstrapping statistics indicated that left inferior longitudinal fasciculus FA significantly mediated the association between *APOE* and *g* (indirect effect = -0.02, 95% C.I's = -0.05 to -0.00) and Block Design (indirect effect = -0.25, 95% C.I's = -0.63 to -0.06), g_{speed} (indirect effect = -0.03, 95% C.I's = -0.07 to -0.01), Digit Symbol Coding (indirect effect = -0.30, 95% C.I's = -0.79 to -0.05), Inspection Time Total (indirect effect = -0.30, 95%, C.I's = -0.76 to -0.06), but not Matrix Reasoning (indirect effect = -0.06, 95% C.I's = -0.20 to 0.17), Symbol Search (indirect effect = -0.08, 95% C.I's = -0.30 to 0.21), g_{memory} (indirect effect = <-0.00, 95% C.I's = -0.22 to 0.21) or Spatial Span (indirect effect = -0.04, 95% C.I.'s = -0.12 to 0.01). Cases of statistically significant mediation are displayed in Figure 7.1.

Figure 7.1. Three-way associations between *APOE* ε, left inferior longitudinal fasciculus tract integrity and cognitive ability adjusted for age 11 IQ.



Note: All associations are standardized β values; [a]Specific association betas varied here because each mediation test used listwise exclusion criteria and therefore had very slightly different sample N's. **P* < 0.05; ***P* <0.01; ****P* < 0.001. Figures in (brackets) represent associations after adjustment for fractional anisotropy.

7.6. Discussion

7.6.1. Overview

The current study investigated the effects of the *APOE* ε and *TOMM40* rs10524523 ('523)' poly-T repeat gene loci, on cognitive ability around the age of 73 years, both unadjusted and adjusted for age 11 IQ ('cognitive ageing'; Deary et al., 2004). Analyses also formally tested whether significant raw nominal associations were mediated by previously reported associations with white matter tract microstructural integrity in this sample (see Chapter 4; *'...White matter integrity'*).

In terms of cognitive ageing, this report found significant deleterious effects of *APOE* ε4 allele possession on several cognitive tasks, independent of age, gender, childhood IQ and cardiovascular disease history; namely, tests of non-verbal reasoning (Matrix Reasoning), visuospatial ability (Block Design, Spatial Span), processing speed (Digit Symbol Coding, Symbol Search, Inspection Time Total), and memory (Logical Memory total). Accordingly this study also found significant nominal effects on general factors of intelligence, processing speed and memory, constructed with PCA. This study also reports significant protective effects of the *TOMM40* 523 'Short' allele on Spatial Span scores in the whole sample, but this did not survive statistical correction for *APOE* ε genotype, and Letter-Number Sequencing in the *APOE* ε3/ε4 genotype group, but this did not survive correction for cardiovascular disease history. Formal tests of mediation (with bootstrapping; Preacher and Hayes, 2008) showed that the left inferior longitudinal fasciculus significantly mediated associations between *APOE* ε4 and *g*, Digit Symbol Coding, g_{speed}, Inspection Time Total and Block Design scores. This tract did not completely mediate these significant associations; the gene-cognitive associations did not attenuate markedly.

All associations with cognitive ability attenuated when corrected for type 1 error with FDR. Cognitive variables are strongly inter-correlated – as reflected by the general factors of

intelligence, memory and processing speed that were constructed with PCA. Although FDR is less affected by inter-correlated test statistics compared with other type 1 error adjustment procedures (such as Bonferroni; Benjamini and Yekutieli, 2001), this can still make correction for multiple testing overly conservative (Williams and Haines, 2011). All significant raw gene-cognitive associations are interpreted cautiously in this context. These inter-correlations also highlight that the genetic associations with cognitive variables are not independent and may partly reflect a degree of shared variance between these tasks (as indicated by *g*).

7.6.2. Interpretation: APOE ε genotype

A large number of previous studies have examined *APOE* and cognitive ageing, synthesised into a large meta-analysis by Wisdom et al. (2011). On the basis of a large number of studies into *APOE* and non-impaired adult cognitive functioning, they found the strongest effects of ε4 allele presence on episodic memory (e.g. WMS-III scores), information processing speed (e.g. Digit Symbol Coding), executive function (e.g. Trail-Making Test-B) and global cognitive ability (e.g. full-scale IQ). The domains of crystallised intelligence (e.g. Boston Naming Test), attention (e.g. Trail-Making Test-A), visuospatial (e.g. Block Design) and working memory (e.g. Digit Span) did not show significant effects, based on several samples (range = 13 to 56 independent samples). Our results in the current sample (aged around 73 years) align almost perfectly with this.

Luciano et al (2009a; 2009b; as described above) reported relatively specific, circumscribed effects of *APOE* genotype on cognitive ability - adjusted for age 11 intelligence – in the LBC1936 sample at age 70 years. In comparison, the current study reports much more broad deleterious significant (raw unadjusted) effects of the ε4 allele on cognitive ageing here, when the participants are on average around three years older. This

could reflect either 1) greater type 1 error in the slightly smaller age 73 sample, or 2) that *APOE* has a stronger influence on cognitive ability at older ages; these explanations are explored below in order.

It is possible that the sample reported here; around 860 participants with a mean age around 73, is meaningfully different – other than being older - from the sample reported on by Luciano et al. (2009a; 2009b), where roughly 1000 participants were around 70 years old. However both studies had very similar exclusion criteria, e.g. MMSE cut-offs below 24 (indicative of dementia), removal of outliers around 3 standard deviations from the mean. However Chapter 3 ('*ADRB2*….'; published as Lyall et al., 2013) showed that the LBC1936 participants who attended both wave one (70 years) and wave two (73 years) had significantly higher age 11 MHT scores compared with participants that attended wave one only (mean scores = 50.16 vs. 47.62, t = -2.89, P = 0.004). This indicates a degree of selection bias, in the sense that the more cognitively impaired individuals did not return for testing at age 73; however, in that case one would expect a restriction of range in cognitive scores, which would typically result in a lower likelihood of spurious results. For these reasons, spurious results here (vs. Luciano et al. 2009a; 2009b) are unlikely.

The different findings by Luciano et al. (2009a; 2009b) and the current study may reflect that *APOE* affects age-related processes that impact on cognitive ability more into older age. These processes could include the maintenance and repair of myelin, neuronal membrane and synaptic connections, among others (Reivang et al., 2013). As an example, Schiepers et al. (2012) found that *APOE* associated with significant cognitive change across later life in the Lothian Birth Cohort 1921, (LBC1921), a sample of community-dwelling older adults. They examined 187 participants assessed at ages 79, 83 and 87 on Verbal Fluency, Logical Memory and Raven's Progressive Matrices (a reasoning test). They found significant interactions between time point and *APOE* genotype, whereby possession of the

ε4 allele was associated with worse Logical Memory (parameter estimate = -0.50, 95% C.I's = -0.93 to -0.07) and Raven's Progressive Matrices scores (parameter estimate = -0.27, 95% C.I's = -0.52 to -0.02), adjusted for age 11 IQ and cardiovascular disease history. The age-related effects of *APOE* may be through influencing risk of cardiovascular disease (which can contribute to cerebrovascular pathology), amyloid beta clearance and neuronal/myelin survival (Smith, 2002; Morris et al., 2010). The first explanation is unlikely to explain the results here because the majority of significant associations survived correction for cardiovascular disease history (although self-reported history – as used here - may not be accurate). It is not possible to distinguish or elucidate the influence of other explanations without further relevant data (this is explored below in 'future research').

The current study is relatively novel in demonstrating that significant associations between *APOE* and cognitive ageing are formally mediated by white matter tract integrity, possibly reflecting the fact that relatively few studies have the requisite genetic, imaging and cognitive data (Salthouse, 2010). This is important because it helps to elucidate the neural substrates underpinning the association between *APOE* and cognitive ageing. Previous chapters have shown that *APOE* is not significantly associated with hippocampal volume, white matter lesions or cerebral microbleeds in LBC1936. This report found that the associations between *APOE* and *g*, Digit Symbol Coding, g_{speed}, Inspection Time Total and Block Design did not attenuate (to non-significance; $P > 0.05$) when variation in left inferior longitudinal fasciculus FA was controlled for, with Boostrapping.

It is plausible that the left inferior longitudinal fasciculus at least partly mediates specific *APOE*-cognitive ageing associations. The inferior longitudinal fasciculus is an occipito-temporal tract (Catani and de Schatten, 2008). Our findings fit in well with a large, relatively similar study by Ryan et al. (2011), who examined whether correlations between FA and cognitive ability differed depending on *APOE* genotype (healthy older adults, N =

126; mean age ranges = 71.6, 69.2 and 67.0 for non-carriers, ε4 carriers and ε4/ε4 homozygotes). They constructed general 'executive' and 'memory' composites based on a range of standard WAIS-III and WMS-III tests, plus the California Verbal Learning and the Wisconsin Card Sorting tests. They found significant differences in correlation strengths for *APOE* ε4+ and ε4- groups, between frontal lobe FA with executive scores (r = 0.46 vs. 0.04) and memory scores (r = 0.43 vs. 0.14), and for temporal stem FA with executive scores (r = 0.50 vs. 0.04) and memory scores (r = 0.57 vs. 0.14; all $P < 0.05$). Westlye et al. (2012a) posited that the stronger correlations between white matter integrity and cognitive ability in *APOE* ε4 carriers may reflect a "computational bottleneck" whereby lowered integrity limits the amount of information that can be transmitted between brain structures, strengthening the FA-cognitive correlation (Westlye et al., 2012a; pp. 514). This provides a reasonable biological explanation for the mediating role of the inferior longitudinal fasciculus and performance on specific cognitive tasks. It is also possible that the integrity of this tract correlates significantly with the actual mechanistic brain imaging phenotype, which was not examined here (Salthouse, 2010). Our findings suggest that a large scale, hypothesis-free examination of *APOE* and cognitive ageing – with a range of brain imaging phenotypes – is required.

7.6.3. Interpretation: TOMM40 '523' poly-T repeat genotype

The author is aware of three previous independent studies of *TOMM40* 523 genotype and cognitive ability, by Caselli et al. (2012), Johnson et al. (2011), and Hayden et al. (2012). Caselli et al. examined 639 non-demented older adults that had been recruited between ages 21 and 97 years (mean ages of genotype groups ranged from 57.8 to 60.9 years), and undergone repeat testing at least once since (mean duration of follow-up = 6.1 years ± 3.1). They examined the effects of *TOMM40* 523 in the whole sample, and in the 'neutral' ε3/ε3

genotype subgroup on Auditory Verbal Learning Test delayed scores. They reported a significant deleterious effect of the VL/VL group in comparison with S/S, whereby the former did not show a test-retest improvement over follow-up (P = 0.04; in *APOE* ε3/ε3 subgroup). They did not find an additive VL allele effect, however. Johnson et al. (2011) examined 117 older adults with the ε3/ε3 genotype, controlling for age, gender, family history of AD and hypertension. They compared participants on AVLT scores. They reported a significant deleterious effect of the VL/VL genotype compared with S/S on total correct scores (means scores = 55.13 vs. 49.63, P = <0.05). Johnson et al. however reported no effects of *TOMM40* 523 genotype on verbal or performance IQ scores on the WAIS-III. Hayden et al. (2012) examined 82 older adults with the *APOE* ε3/ε3 genotype (mean age = 80.6, SD = 6.0) in terms of *TOMM40* 523 genotype and a broad battery of memory and intelligence tests. Controlling for age, gender and education, they found a significant deleterious effect of the S/VL genotype (vs. S/S and VL/VL genotypes) on Paired Associates Learning and Verbal Recall memory scores, Rapid Visual Information Processing latencies, and Digit Span scores (P value range = 0.016 to 0.48). They had no metric of prior cognitive ability, however.

The above findings contrast with the current study in that here the present results showed no significant effects of *TOMM40* 523 genotype, independent of *APOE* or cardiovascular disease history. This suggests that previous studies may to an extent report type 1 errors; note the relatively marginal significance reported by Caselli et al. (2012), and the relatively small samples reported by Johnson et al. (2011) and Hayden et al. (2012).

7.6.4. Future research and limitations

Future studies may investigate the effects of *APOE* and *TOMM40* genotypes on longitudinal cognitive change in this sample. The current study examined cognitive ability at around age 73. Participants also have similar data at age 70 (Luciano et al. 2009a/2009b reported generally modest cross-sectional effects of *APOE* genotype on cognitive data) and are currently undergoing repeat testing (around age 76). Tests of association with *APOE* or *TOMM40* genotypes may be more sensitive to later-life cognitive change compared with cross-sectional abilities. Schiepers et al. (2012; described above) for example showed significant cognitive change in the LBC1921 longitudinally across older age (79-to-83-to-87 years).

This study reported generally modest mediation of significant *APOE*-cognitive ageing associations via white matter tract integrity. In this regard, future studies may investigate the role of other brain imaging phenotypes that were not examined in this thesis. Interesting phenotypes could include subcortical brain structures such as the amygdala, or dorsolateral/orbitofrontal cortical volumes for example (Pievani et al., 2009).

This chapter took a 'causal-step' approach of examining associations between *APOE/TOMM40* with brain imaging and cognitive ageing phenotypes (Hayes, 2009). This dictates that:

1. The total effects of *APOE/TOMM40* on brain imaging and cognitive variables must each be statistically significant ($P < 0.05$).
2. The association between imaging and cognitive variables must remain statistically significant controlling for *APOE/TOMM40.*
3. Having corrected for variation in the mediating imaging variables, the association between genetic and cognitive variables must either attenuate to non-significance ('fully-

mediated' model; i.e. $P > 0.05$) or attenuate in strength, but not necessarily to non-significance ('partial' mediated model; still $P < 0.05$; MacKinnon et al., 2002).

This report examined variables that were statistically significantly inter-correlated at $P < 0.05$, and where mediation would therefore be most likely. A limitation of this 'causal steps' approach is that each hypothesis test carries a possibility of type 1 or type 2 error. Rather, Hayes (2009) suggests hypothesis-driven testing of indirect effects, regardless of the statistical significance of the mediator's association with the independent and dependent variables; it is possible that significant *APOE*-cognitive ageing associations occur via very small effects on a large, distributed range of brain imaging phenotypes. Future studies should consider a large-scale, hypothesis free examination of *APOE* genotype, different brain imaging phenotypes and cognitive ageing variables.

7.6.5. Summary

The current study investigated the independent effects of the *APOE* ε and *TOMM40* 523 poly-T repeat gene loci on cognitive ageing, and the extent to which nominally significant gene-cognitive associations were mediated by previously reported genetic associations with white matter tract integrity in the LBC1936 (around the age of 73 years).

APOE ε - but not *TOMM40* 523 - genotype was significantly associated with performance on several different measures of cognitive ability, including general factors of intelligence, information processing speed and memory, and more specifically Block Design, Digit Symbol Coding, Symbol Search, Inspection Time Total, Spatial Span and Logical Memory total scores (raw P values all < 0.05). These significant associations were independent of age 11 IQ and cardiovascular disease history.

Formal tests of mediation showed that several *APOE*-cognitive ageing associations were significantly mediated by integrity of the left inferior longitudinal fasciculus; however,

the associations themselves did not attenuate markedly when FA of this tract was statistically controlled for. A range of brain structural imaging phenotypes - including matter microstructural integrity - may form the anatomical basis for significant associations between *APOE* genotype and cognitive ageing.

Chapter 8: Thesis overview and discussion

8.1. Review of background and findings

The *APOE* ε locus has previously been statistically significantly associated with increased risk of Alzheimer's disease (AD), and this association has been replicated in a number of independent samples (e.g. Corder et al., 1994; Farrer et al., 1997; Genin et al., 2011). However the association between *APOE* ε4 allele presence and AD is not completely penetrant; it accounts for around 4-5% of the variation contributing to liability for AD. This suggests it may be modified by other variables, including other genetic loci (Lee et al 2013). More recently, variation in the *TOMM40* '523' poly-T repeat locus has been reported to significantly influence age of AD onset independently of *APOE* genotype, although the statistical significance and directions of these associations have varied (Roses et al., 2010; Crenshaw et al., 2013).

Using the Lothian Birth Cohort 1936 (aged around 73 years; LBC1936), the analyses in this PhD thesis had three main aims. The first two aims were to quantify the independent effects of the *APOE* ε status and *TOMM40* 523 loci on specific structural brain magnetic resonance imaging (MRI) phenotypes; namely white matter microstructural integrity, hippocampal volumes, white matter lesions, and cerebral microbleeds. The subsequent third aim was to formally investigate the anatomical substrates of genetic associations with cognitive ageing.

There were several previous studies of *APOE* ε and brain structural/cognitive phenotypes in community-dwelling, generally healthy older adults, but few-to-none for *TOMM40* 523. Specifically, studies reported deleterious effects of the *APOE* ε4 allele; however, directions varied for *TOMM40* 523 in terms of showing either deleterious or protective effects of the 'Short' allele (vs. Long and Very-long alleles). Reflecting that, this thesis predicted independent significant associations in the deleterious direction of the *APOE*

ε4 allele (vs. ε3/ε3 or ε4-absent groups), but with no specific direction for *TOMM40* 523 genotype.

In the context of a relatively large number of tests of association (and hence increased risk of type 1 error), results showed that possession of *APOE* ε4 or *TOMM40* 523 Short allele variants (vs. absence) were independently significantly associated with the microstructural integrity of specific white matter tracts, but not white matter lesions, hippocampal volume or cerebral microbleeds. These significant associations were 1) statistically independent in the sense that effects of *TOMM40* 523 survived correction for *APOE* ε4 presence, and 2) biologically independent in the sense that the *TOMM40* 523 effect was statistically significant in a subgroup of participants with the *APOE* ε3/ε4 genotype (i.e. cannot be attributed to variation in *APOE* genotype; Roses et al., 2010). *APOE* ε4 - but generally not *TOMM40* 523 - was significantly associated with worse cognitive ageing, and these associations were partially mediated by the integrity of specific white matter tracts. The reported significant associations with white matter integrity and cognitive ageing survived correction for cardiovascular disease history. This indicates that it is less likely that effects of *APOE/TOMM40* on age 73 brain variables are secondary to associations with cerebrovascular pathologies such as stroke.

The majority of associations were non-significant when corrected for multiple testing with the False Discovery Rate (FDR; Pike, 2011). Type 1 error correction could be considered overly conservative when testing significantly inter-correlated brain imaging/cognitive phenotypes because each test is not independent (Nyholt, 2001). FDR is less affected by inter-correlated test statistics compared with other adjustment procedures (Benjamini and Yekutieli, 2001). All significant raw associations are interpreted cautiously in this context, and this issue is discussed further in '*Limitations'*.

8.2. Relation to predictions

The study presented in Chapter 4 found significant deleterious independent effects of *APOE* ε4 and *TOMM40* 523 on specific white matter tract integrity variables, and a significant deleterious effect of *APOE* ε4 on a large proportion of cognitive ageing variables. ('Cognitive ageing' reflects test scores at age 73 adjusted for age 11 IQ; Deary et al., 2004). Those findings aside, generally the *a priori* predictions were not met: other than Chapter 4 there was no effect of *APOE* ε on the majority of imaging phenotypes, or *TOMM40* 523 on imaging/cognitive phenotypes. Analyses therefore generally failed to support previous claims drawn from smaller studies.

8.3. Integration with previous reports

Specific common gene loci typically account for only small amounts of variation in complex brain-related phenotypes (e.g. cognitive abilities). Single candidate loci often fail to replicate across studies for different reasons including insufficient power, study design/analytic strategy and low prior probability of true biological association (Hattersley and McCarthy, 2005).

Previous studies have investigated *APOE* and the imaging phenotypes described, and in some instances describe statistically significant associations where in the present analyses they were null (Chapters 5-7) or more specific and restricted effects than previously reported (Chapter 4; Gold et al., 2012). In several cases previous reports had observable limitations, and there are general and study-specific reasons why these reports may have shown spurious results:

1. Chapters 4-7 observed that previous studies of *APOE/TOMM40* do not always control for important covariates such as cardiovascular disease history (Wisdom et al., 2011), and

several reports examine samples with wide age ranges. First, this is important because *APOE* in particular has been significantly associated with greater risk of cardiovascular and more specifically cerebrovascular diseases, which could directly contribute to brain changes (Ward et al., 2009). In this LBC1936 sample, there were no significant genotype-phenotype associations which attenuated to non-significance when corrected for cardiovascular disease history; however this may not be the case in independent samples. Second, this is important because while studies typically statistically adjust for the effects of age, the correlation between age and brain phenotypes is unlikely to be completely unique and rather may be via several processes *associated* with chronological age; it is therefore unlikely that statistically controlling for age would completely capture this (Hofer and Sliwinski, 2001). These factors could result in spurious results because several brain imaging/cognitive phenotypes show change with age and in the presence of cardiovascular/cerebrovascular diseases; effects of *APOE* or *TOMM40* 523 could occur via these variables if not adequately controlled (Wardlaw et al., 2011).

2. The majority of studies were also relatively small (i.e. N < 150, typically) and therefore less likely to be reliable because they are less representative of the general population (Wacholder et al., 2004). This is particularly applicable to previous studies of *APOE* and white matter microstructural integrity (Chapter 4), where only Westlye et al. (2012b) reported on a sample N over 150. A practical example is in Chapter 3 (Lyall et al., 2013; *'ADRB2…'*). In 162 members of the LBC1936 that had by that time undergone diffusion tensor-MRI (simply 'DTI'), Penke et al. (2010b) reported a significant effect of the rs1042714 SNP in the *ADRB2* gene ($\beta = 0.16$, $P = 0.043$). When examined again in the larger, full LBC sample (Chapter 3), the association had attenuated to non-significance ($\beta = 0.01$, $P = 0.803$, n = ~765).

3. In the case of *APOE* and hippocampal volume (Chapter 5), two large previous studies that reported significant associations (Den Heijer et al., 2002; Lemaitre et al., 2005) did not statistically adjusted for general brain volumetric atrophy. This is important because MacLullich et al. (2002) described significant inter-correlations between subcortical brain structural and general brain tissue volumes; this means it is possible that previous significant associations were between *APOE* and general brain atrophy rather than hippocampal volume specifically. Furthermore, Den Heijer et al. (2002) examined hippocampal volume as a ratio proportion of intracranial volume (maximal head size). This approach is limited because any significant effect of *APOE* on hippocampal volume could reflect an effect on intracranial volume only, because this would also affect the 'ratio' score. Indeed, further analysis in Chapter 5 showed that 1) *APOE* genotype significantly associated with the 'ratio' variable, but with not hippocampal volume statistically adjusted for intracranial volume as a model covariate, and 2) the 'hippocampal ratio' variable was statistically significantly correlated with intracranial volume in mm^3.
4. In the case of *APOE* and cerebral microbleeds (Chapter 6), previous large (significant, positive) studies have not differentiated between 'certain' and 'uncertain' microbleeds as was done here. These studies may be including brain microbleed mimics which do not necessarily reflect deleterious processes, such as cortical vessels or flow voids (Cordonnier et al., 2011).

In the case of *APOE* and white matter lesions (Chapter 6), the null association corresponds well with a large meta-analysis (Paternoster et al., 2009) suggesting a truly null association, when large amounts of data are collated.

There have been previous studies of *TOMM40* 523 genotype, cognitive ability, and hippocampal volumes. These are generally inconsistent; reporting relatively small samples

(Johnson et al., 2011; Hayden et al., 2012), or very marginal statistical significance, uncorrected for multiple testing (Caselli et al., 2012). The findings in the LBC1936 regarding *TOMM40* 523 are therefore not contradicting a considerable literature.

8.4. Contribution to the literature

8.4.1. Anatomical basis of APOE-cognitive ageing associations

In Chapter 7, it was reported that integrity of a specific white matter tract – the left inferior longitudinal fasciculus – significantly mediated part of the association between *APOE* and specific cognitive abilities, particularly related to information processing speed (e.g. Digit Symbol Coding and Inspection Time Total tasks). Few studies have investigated the anatomical substrates of genetic contributions to cognitive ageing with mediation (as detailed below). Specifically, this thesis contributes to understanding of the anatomical basis of *APOE*-cognitive ageing associations.

Large samples of healthy adults with genetic, brain imaging and cognitive data are rare, and as such there are few studies that examine mediation or structure-ability correlations (e.g. Honea et al. 2009; Salthouse, 2010). To the author's knowledge, the largest previous study was conducted by Espeseth et al. (2006), who examined 230 older adults with an age range of 53 to 75 years. Participants completed the Wechsler Abbreviated Scale of Intelligence, California Verbal Learning Test, a cued visual discrimination test of reaction time, and underwent detailed brain MRI. Carriers of *APOE* ε4 showed significantly slower average reaction times (F [1, 222] = 6.32, P = 0.013), and lower white matter volume although this did not attain statistical significance (F [1, 93] = 2.71, P = 0.100; vs. non-carriers). They tested the extent to which white matter volume mediated the *APOE*-cognitive association. An ANCOVA controlling for white matter volume (and age), showed a main

effect of white matter volume (F [1, 92] = 4.05, $P < 0.05$), but not *APOE* genotype. This attenuation indicates that the association between *APOE* and reaction time was partly dependent and at least in part mediated by white matter – although Espeseth et al. did not formally test this. The current sample adds significant data to this – in terms of participant sample size and range of cognitive/brain imaging phenotypes examined.

White matter tracts form the basis for connected brain networks, providing a direct plausible foundation for cognitive functioning (Penke et al., 2012). Lower tract integrity may result in a processing 'bottleneck' that hinders efficient information processing (Westlye et al., 2012a). The inferior longitudinal fasciculus is an occipito-temporal tract (Catani and Thiebaut de Schotten, 2008). The inferior longitudinal fasciculus tract may mediate the transfer of visual signals from visual areas to anterior temporal regions (Catani et al., 2003), although understanding of its relevance to specific cognitive abilities - independent from other white matter tracts - is limited (Ashtari, 2012).

A study by Penke et al. in a subset of the LBC1936 sample (2010a) showed that a general factor of white matter integrity (constructed with principal components analysis; 'g_{FA}') was significantly associated with a general factor of information processing speed (g_{speed}; r = -0.24, P = 0.007; n = 132), and explained 10% of the variance in general cognitive ability ('*g*'; n = 420, Penke et al., 2012). Penke et al. (2012) noted that no single tract showed significant associations with general cognitive ability beyond what was explained by the general factor g_{FA}, reflecting that a large proportion of variance in white matter integrity is shared, emphasising that tracts probably function together, and warning against placing too much emphasis on associations found with only one or a few tracts.

As discussed in Chapter 4, it is unclear why *APOE* genotype would be significantly associated with the left inferior longitudinal fasciculus in particular, over and beyond other tracts. There is biological rationale for why white matter tract integrity may affect cognitive

ability however this is not necessarily at the tract-specific level. The reported mediation may be either mechanistic in some way, or reflect a secondary correlation with a brain marker that is linked to this tract. It is possible that the effects of *APOE* on cognitive ageing are very subtle and mediated via a large range of brain imaging phenotypes. It should also be noted that – taking the thesis as a whole - Chapter 7 reflects the end point of a large number of tests of association (including *APOE*/*TOMM40* and imaging/cognitive phenotypes, and their inter-correlations), each of which will have a natural possibility of type 1 or type 2 error (Hayes, 2009). Chapter 7 tested for mediation only in cases of significant nominal three-way associations between genetic/imaging/cognitive variables at $P < 0.05$, where mediation would be most likely. A hypothesis free approach to investigating the anatomical substrates of *APOE*'s contributions to cognitive ageing - where simply the indirect 'effects' (reflecting the extent of statistical mediation) are examined regardless of the strength or significance of genotypic/phenotypic associations – may be fruitful (Hayes, 2009). Overall it is likely that a range of brain imaging variables – including white matter microstructural integrity - partly mediate associations between *APOE* and cognitive ageing.

8.4.2. A large amount of homogenous data that addresses several previous methodical issues

A number of previous reports have examined *APOE* and structural brain imaging phenotypes with MRI, and the majority of these studies are in relatively small samples (i.e. N < 150; Gold et al., 2012). The smaller studies are less likely to be reliable (Flint and Munafo, 2012). Chapters 4-7 therefore provide a relatively large amount of new data in community-dwelling older adults. There is reason to believe the data here are of generally high quality relative to previous reports in terms of testing a relatively large imaging/cognition/genetics sample with relatively narrow age ranges, detailed demographic and medical phenotyping, and a measure

of childhood intelligence, as detailed in each chapter and above in '*Integration with*…'. Also detailed above (in '*Integration with*…') are several study-specific limitations to some larger previous reports, which have been addressed here. Thus, the high-quality data presented here contribute significantly to the literature in terms of addressing previous methodological issues.

Chapter 4 examined white matter tract integrity using a different methodology to that used previously. Previous studies of *APOE* and white matter integrity used Tract-based spatial statistics (TBSS). Chapter 4 in contrast used probabilistic neighbourhood tractography, an approach for automatic and reproducible tract segmentation, as implemented in the TractoR package for fibre tracking analysis (Clayden et al., 2011; Tractography with R website, 2012). This method segments the same fasciculus-of-interest in different individuals by identifying the best-matched tract from a group of 'candidate' tracts that most closely resembles a predefined reference tract in terms of both length and shape. This approach has advantages over tract-based spatial statistics in that each tract is segmented in native rather than standard space, therefore providing a better representation of specific tract anatomy in each individual (Bastin et al., 2013) preserving the individual variance of interest. The tracts are also visually inspected and corrected/rejected where processing has failed. It is also optimised for older subject's brains which commonly show pathological changes. This contributes to the *APOE*/white matter microstructure literature by adding a large amount of high quality data, with an imaging analysis methodology with several possible advantages over previous methods.

8.4.3. TOMM40 poly-T repeat genotype is independently associated with white matter tract integrity but not cognitive ageing, hippocampal volume, white matter lesions or cerebral microbleeds

The *APOE* locus has a well replicated association with AD, and it is important to understand modifiers of that risk which may extend to non-pathological cognitive ageing – in this case *TOMM40* 523 poly T repeat length genotype. Previous studies have varied in terms of showing a statistically significant - or null - association between *TOMM40* 523 and AD disease risk, or age of onset (Roses et al., 2013; Jun et al., 2012). The exact direction of association has varied in terms of the Short allele showing a deleterious (e.g. Cruchaga et al., 2011) or protective effect (e.g. Roses et al., 2010).

In a relatively large imaging genetics sample, we found deleterious effects of the *TOMM40* 523 Short allele on white matter tract microstructural integrity independent of *APOE* genotype; but ultimately not hippocampal volume, white matter lesions, microbleed or (generally) cognitive ageing phenotypes. This may reflect two points:

1) There may be a degree of brain plasticity which enables carriers of the *TOMM40* 523 Short allele to have significantly worse white matter integrity (as reported in Chapter 4), but preserved cognitive ability (…Chapter 7). As an illustrative example, Bendlin et al. (2008) examined 36 adults (mean age = 28.37, SD = 9.78) that had traumatic brain injuries (TBI; confirmed with computerised topography on the same day), and compared them with 19 age-and gender-matched controls. Participants underwent detailed cognitive and brain imaging assessments around 2 months after injury, and around 1 year later. Controlling for age, gender and intracranial volumes where applicable, they found that the TBI group had significantly lower fractional anisotropy (FA) and higher mean diffusivity (MD) reflecting worse integrity in several white matter tracts (exact statistics not shown), and lower cognitive ability on different memory and executive function tests (e.g. California Auditory Verbal

Learning test and Digit Symbol Coding). At follow-up assessment, they found that the TBI group showed significantly greater declines in FA/increases in MD in several tracts (possibly reflecting Wallerian degeneration after the initial TBI insult; Bendlin et al., 2008). However several regions showed significant decreases in MD (reflecting improved integrity); namely the internal capsule, superior/inferior longitudinal fasciculi, corona radiate, and the anterior thalamic radiation. Similarly, performance on several cognitive tasks improved over the two time points on most tasks (e.g. Digit Symbol Coding standardized mean score = 7.7, SD = 2.9, vs. 44.4, SD = 12.9, P <0.05). This could indicate a degree of plasticity in how brain architecture subserves cognitive ability, meaning that the integrity of specific white matter tracts may not have an absolutely direct association with non-pathological cognitive ageing (Bendlin et al., 2008).

2) White matter microstructure assessed with DTI may be more sensitive to genetic variables than other imaging or cognitive phenotypes examined in this thesis. For example, in the above report by Bendlin et al., at Timepoint 2 (around 1 year after TBI) white matter volume assessed with T1-weighted images was far less sensitive to showing significant differences between TBI/control groups, compared with FA/MD as assessed by DTI (which was more sensitive). Specifically, significant differences ($P < 0.05$) were found in 1.79% of FA voxels and 3.91% of MD voxels, compared with 0.04% of total T1 white matter voxels. DTI white matter metrics may be a more sensitive phenotype to insult (or 'risk' genotypic variants) compared with gross white matter loss or structural volumetric atrophy (Paorpaoli et al., 2001).

The findings in this thesis progress understanding of *TOMM40* 523 and cognitive/brain imaging phenotypes by demonstrating significant associations specifically with white matter integrity, but not other structural brain or cognitive ageing phenotypes. This is important because it may help to elucidate the possible anatomical mechanisms

underlying association between *TOMM40* 523 and AD, in healthy older brains with no evidence of the disease. Future studies in independent samples will help to elucidate – and replicate – similar effects of *TOMM40* 523, independent from *APOE*.

8.5. Implications

8.5.1. Future candidate gene studies should be carefully controlled; standardised criteria may be required

Imaging genetics is based on the premise that genetic variation will contribute more directly to phenotypic variation at the biological and brain structural/functional level, compared with at the level of complex behavioural traits such as cognitive ability (Flint and Munafo, 2012). It is unlikely that single common genetic variants account for more than small amounts of variance in cognitive ability (see Davies et al., 2011; Munafo et al., 2006). Studies of specific genetic variants often do not replicate consistently (Munafo et al., 2006) and it would be beneficial to limit inter-study heterogeneity. An important implication of Chapters 4-7 is that imaging genetic research may benefit from more specific universal guidelines in terms of methodology and analysis.

Journals are increasingly setting guidelines for candidate gene studies. For example Hewitt (2012) describes guidelines for the journal *Behavior Genetics*: the study must be adequately powered; any novel findings must meet the equivalent of genome-wide significance taking into account multiple testing and valid covariates. Further broad recommendations are described by Little et al. ('STREGA' criteria; 2009); studies should define laboratory methods; how any biases were addressed; software; Hardy-Weinberg statistics; multiple testing correction; numbers of individuals that failed genotyping; relevant demographic/clinical information grouped by genotype. For comparison, Maxwell et al.

(2011) provide a detailed checklist for considering the quality of imaging genetics papers, including consideration of; study size; imaging variables clearly defined (e.g. microbleed presence vs. absence); number of independent imaging raters; inter/intra-observer agreement?; blinding of imaging/genetics staff to participant information. Maxwell et al. rated ten studies of candidate genes and microbleeds, out of a maximum possible score of ten. The average score was 4.8, with a range of one to nine. Maxwell et al. note that in many cases studies simply did not report these. This implies significant inter-study heterogeneity that could be addressed *a priori* and that certain details should be stated as standard across studies. This is important because single common gene variants account for only small amounts of variance and therefore may be sensitive to differences in data collection/analysis (Hattersley & McCarthy, 2005). Relatively standardised reporting would make it easier to synthesise the state of the literature (Maxwell et al., 2011; Wardlaw et al., in press).

8.5.2. Should TOMM40 poly-T repeat genotype be considered as standard in studies of APOE ε?

The significance of the *TOMM40* 523 poly-T repeat is an area of on-going epidemiological and biological research. Roses et al. (2010) noted that while the *APOE* ε4 allele is in very strong linkage with the *TOMM40* 523 Long allele (specifically around 98%), with ε3 linked to either Short or Very-long. Beyond the obvious question of what effect each allele has on brain-related phenotypes such as cognitive ageing or brain structure, *TOMM40* 523 genotype may add additional noise to studies of *APOE* ε. Specifically, the *TOMM40* 523 Short allele (vs. Very-long) may modify the effect of *APOE* ε3 for some phenotypes (e.g. Chapter 4, *'...white matter integrity'*) and this may partly account for any inconsistencies in reports of *APOE* ε. In previous reports of *APOE*, the allele frequencies and linkages for *TOMM40* 523 –

relative to *APOE* ε2 or ε3 alleles - are not clear. Future reports should consider controlling for this – perhaps by ensuring frequencies are relatively similar across studies.

8.5.3. The effect of APOE ε4 does not appear to reflect the TOMM40 'Long' allele

The *TOMM40* 523 'Long' allele is in significant linkage with the *APOE* ε4 allele (Linnertz et al., 2012; Chapter 2; '*Methodology'*). There is biological rationale for why variations in these gene loci may contribute to phenotypic differences, respectively via lipid transport and mitochondrial translocase processes (Hardy, 2006; Swerdlow et al., 2010). Roses et al. (2010) argue that significant associations between *APOE* ε4 and brain-related phenotypes may be reflective of the *TOMM40* 523 L allele (Roses et al., 2010). However in Chapter 7 we found a clear divergence where *APOE* ε but generally not *TOMM40* 523 genotype was associated with cognitive ageing at age 73. This suggests that *APOE* ε4 is not simply reflecting a causal role of *TOMM40* 523 in this sample (although note that *APOE* ε may simply be in linkage with a true 'causal' variant).

8.6. Thesis strengths

8.6.1. The Lothian Birth Cohort 1936 dataset

The LBC1936 sample has childhood cognitive ability, genetic, sociodemographic, medical, and detailed cognitive and brain imaging information; it is rare in having the requisite data to examine the brain imaging substrates of genetic contributions to cognitive ageing (Salthouse, 2010). The sample has a narrow age range (mean age = ~73 years, SD = ~0.7) and is relatively large for a brain imaging study which makes it more likely to be reliable compared with smaller studies. Structural MRI phenotypes are intuitively closer to the biology of

genetic variation compared with cognitive ability because they are not as affected by state-dependent variables such as fatigue (Penke et al., 2010b).

The current sample is drawn from community-dwelling older people who are relatively healthy (Wardlaw et al., 2011; Deary et al., 2007; 2012). As detailed in Chapters 3 and 6, this sample primarily consisted of community-dwelling older adults that varied in terms of overall health (e.g. prevalence of Stroke or Hypertension). Cardiovascular disease prevalence frequencies are comparable to other large studies of ageing, such as the Rotterdam Study of Cognitive Ageing (Vernooij et al., 2008; See Chapter 6, Table 7.3). The *APOE* ε and *TOMM40* 523 genotypes were both in Hardy-Weinberg equilibrium, indicating relatively similar frequencies to what would be predicted in a general population (see Chapter 2 for relevant statistics). It is worth noting, however, that the LBC1936 sample is slightly restricted in range, towards the upper-end of general intelligence and socio-economic status (Deary et al., 2012; also see below).

8.7. Thesis limitations

There are possible general- and chapter-specific limitations to the empirical chapters in this thesis, and these are examined here in that order.

8.7.1. A lack of age-related differentiation?

The brain imaging and cognitive phenotypes investigated here fundamentally show age-related change (e.g. Raz et al., 2012; Wisdom et al., 2011). It is possible that the current sample of non-demented, generally healthy older adults, around 73 years old, have not undergone sufficient age-related change to show significant differentiation according to genotype in terms of some phenotypes like hippocampal volume. Evidence from Chapter 7

makes this seem unlikely: *APOE* genotype was significantly associated with a range cognitive test scores at age 73, but less so at age 70 when examined in separate reports by Luciano et al. (2009a; 2009b). However it may still be the case that effects of the *APOE/TOMM40* 523 gene loci are more pronounced at older ages, and this could be examined when cognitive/imaging data on the LBC1936 participants aged ~76 years are available (i.e. when repeat longitudinal testing is completed).

8.7.2. Selection bias

Non-random selection bias occurs when a sample of participants vary from being truly random for a specific reason (Berk, 1983). This could be important if this reason was relevant to cognitive ability. Generally, the LBC1936 sample is slightly restricted in range in certain ways. Firstly, the sample is generally of restricted social class (mean = 2.4, SD = 0.9; generally 'Managerial & Technical' to 'Skilled' occupations; Deary et al., 2012). Secondly, the LBC1936 sample is of a relatively higher childhood intelligence (mean MHT score = 49.0, SD = 11.8, N = 1091), compared with the general population (mean score = 36.7, SD = 16.1; Cohens *d* = 0.87; 'large effect'; Deary et al., 2012). These restrictions of range could mean that the LBC1936 sample may slightly underestimate any true effect sizes.

8.7.3. Lack of intermediate longitudinal data

The current study primarily examines cognitive and genetic phenotypic variables in the LBC1936 as assessed around age 73 ('Wave 2'). In certain analyses, these variables are statistically adjusted for age 11 intelligence as assessed with the Moray House test (no.12), in order to better reflect 'cognitive ageing'. This childhood intelligence measure is a rare and valuable piece of information, reflective of cognitive ability before the effects of age-related changes due to stroke, injuries etc (Deary et al., 2007).

APOE moderates lipid metabolism, including the development and maintenance of myelin (that characterizes white matter), and of neuronal membrane (Bu, 2009). This means that *APOE* could play a role in brain development from age 11 to early adulthood, and significant associations with cognitive ageing are at least partly reflective of this. Further intelligence data from around adolescence would inform this possibility.

8.7.4. Correction for multiple testing

A large number of tests of association were performed in this thesis and this increases the risk of type 1 error, as inherent with multiple testing. However, the association tests were primarily not independent because brain/cognitive variables were often statistically significantly inter-correlated at $P < 0.05$; this can make correction for multiple testing overly conservative (Nyholt, 2001; Williams and Haines). As such, this thesis corrected for multiple testing with the FDR (which is less conservative than Bonferroni correction; Benjamini and Hochberg, 1995), but cautiously considered nominally significant results further, with the caveat that many attenuated when adjusted for FDR. This was to avoid type 2 errors. These considerations highlight that the results of this thesis require independent replication in large

samples; this would help to determine the extent to which the nominally, raw significant results reported in this thesis are spurious, if this is the case (Williams and Haines, 2011).

8.7.5. Specific MRI imaging analytic limitations

Chapter 4 used probabilistic tractography to examine white matter tract integrity. While having advantages (described above), it does not allow a hypothesis-free voxelwise examination of white matter integrity in the entire brain. This is important because previous studies have reported significant deleterious effect of *APOE* ε4 possession in regions of the brain that were not assessed here, e.g. the fornix (Smith et al., 2010). This is not a critical limitation because it does not detract from the key conclusion that nominally significant raw effects were weaker and more specific that would be expected on the basis of previous, smaller papers (see Gold et al., 2012 for a review).

8.8. Future research

8.8.1. Instrumental variables

This thesis reported significant effects of *APOE*/*TOMM40* 523 genotypes on some imaging and cognitive phenotypes but not others. *APOE* plays a role in moderating lipid metabolism which is relevant to amyloid beta plaque levels (Cramer et al., 2012). *TOMM40* encodes for a translocase of outer-mitochondrial membrane complex, and poly-T repeat length may be important for *TOMM40* gene expression (Bekris et al., 2011) and mitochondrial function (Billing et al., 2011). Associations with cognitive decline/AD may be mediated by these intermediate variables. Chapters 4-7 are therefore slightly limited in that it there are not concurrent *in vivo* measures of relevant intermediate variables such as amyloid-beta accumulations, *APOE*/*TOMM40* expression levels or metrics of mitochondrial function (e.g.

'ΔΨm', reflecting depolarization of transmembrane potential; Hedskog et al., 2012). These data would help to elucidate the instrumental mechanisms through which these gene variants actually affect the brain and cognitive function (Smith and Ebrahim, 2003).

8.8.2. Longitudinal imaging of the LBC1936

There are possible limitations to cross-sectional analyses of brain structure and cognitive ability. There is a large amount of natural inter-individual variation in healthy human brain morphology, and this could either artificially affect the sensitivity of detecting morphological differences across groups (Scahill et al., 2003). Examining within-participant longitudinal age-related changes in brain structure is less susceptible to natural inter-individual morphological variation. As a hypothetical example, two healthy older individuals may fundamentally have significantly differently sized subcortical volumes, but this may not in itself reflect any deleterious processes. A significantly greater rate of longitudinal relative change in older age, however, may be reflective of deleterious processes distinct from normal healthy age-related brain changes (Resnick et al., 2003). (Whilst most studies correct from general head size with intracranial volume, this is not a perfect correction for inter-individual variation in brain morphology; Scahill et al., 2003).

The LBC1936 sample is currently undergoing repeat MRI scanning around age 76 years, using an identical protocol to that at around age 73 (see Wardlaw et al., 2011). Future research may investigate the role of 'risk' genotypes on rates of age-related change in brain MRI phenotypes from ages 73 to 76, and on cognitive change across three waves (~ages 70-73-76).

8.8.3. APOE, TOMM40 and further brain MRI phenotypes

A number of brain imaging phenotypes were examined in this thesis, and each of these were considered informative or relevant for different reasons. There are other brain imaging phenotypes that may be important anatomical substrates of genetic associations with cognitive ageing.

As an example, functional brain imaging may be an important mediating variable. Trachtenberg et al. (2012) examined the effects of *APOE* on functional connectivity with functional MRI, which measures regional cerebral blood flow with a metric called blood oxygen level dependent (BOLD). They examined 76 healthy adults (age range 32 to 55 years). Participants encoded images of houses for a memory task, and were excluded from analysis if their post-scanning score was below 2 standard deviations. Controlling for age, gender and family history of dementia, Trachtenberg et al. found that *APOE* ε4 carriers showed significant increases in activation during the encoding task in four clusters, particularly in parieto-temporal brain regions (vs. the ε3/ε3 genotype; significant Z scores ranged from 2.3 to 4.9). This highlights that different imaging methods are required to elucidate the anatomical substrates of genetic contributions to cognitive ageing. Other research could investigate white matter integrity using different methods such as TBSS (see Chapter 4), or examine sub-divisions of the hippocampal formation (see Chapter 5).

8.8.4. What else modifies the effects of APOE?

There may be variables that moderate the effects of *APOE* and/or *TOMM40*. These could be genetic or environmental, and are detailed below in turn.

Other genetic variants may partly moderate the effects of *APOE* or *TOMM40* 523. For example, Hamilton et al. (2011) investigated several genes that previous GWAS studies had suggested may contribute to AD risk (e.g. Lambert et al., 2010). Hamilton et al. examined their effects on cognitive ability in the LBC1936 at around 70 years of age (N= 1039) and LBC1921 at around age 79 years (N = 453). These genetic variants were in the *APP*, *PS1*, *PS2*, *BIN1*, *CLU*, *CR1* and *PICALM* genes, and in the genomic region surrounding the *BLOC1S3/EXOC3L2/MARK4* genes on chromosome 19. They conducted analyses in *APOE* ε4+ and ε4- groups. Controlling for age, gender and childhood intelligence, they tested the effects of specific SNPs on different cognitive abilities. They found a significant effect of the *TRAPPC6a* locus based on three SNPs with Matrix Reasoning performance in the *APOE* ε4- group, in both LBC1936 and 1921samples (SNPs rs7247764/rs28555639/rs1246041; overall $\beta = -0.20$, $P = <0.001$). They also found significant associations for haplotypes based on SNPs in the *APP* gene, with Logical Memory total scores (rs2829997/rs440666/rs2014146; $\beta = 1.3$, $P = 0.004$, and rs1783025/rs380417/rs1787438; $\beta = 0.72$, $P = <0.001$) in the *APOE* ε4+ group of LBC1936 only.

There is also evidence that important environmental variables may moderate the effects of *APOE*/*TOMM40* 523. For example Haan et al. (1999) examined 5888 members of the Cardiovascular Health Study, a sample of adults that underwent repeat cognitive testing on the Digit Symbol Coding task at least twice during nine years follow-up (baseline age range = 65-85, no means provided). Participants were genotyped for *APOE* ε and were also assessed in detail for clinical or subclinical cardiovascular disease (e.g. with blood pressure readings, atrial fibrillation). Haan et al. found that *APOE* ε4 presence was associated with

significant decline on the Digit Symbol Coding task (unstandardized β = -2.00, $P < 0.05$). However several cardiovascular/cerebrovascular risk factors were also associated with decline such as diagnosis of diabetes mellitus, or high fasting plasma glucose, 2 hour glucose tolerance, systolic blood pressure, or high common/internal carotid artery thickness (all $P < 0.001$). They reported a significant interaction whereby individuals with low ankle arm blood pressure (<0.90 mmHg) and at least one *APOE* ε4 allele had 8.3 times greater decline that subjects with neither risk factor, compared with 2.94 (ε4 only) or 3.66 (low blood pressure only). These findings could possibly reflect the deleterious effects of *APOE* ε4 on brain repair mechanisms, resulting in a synergistically greater effect of cerebrovascular risk factors on cognitive change (Lee et al., 2008). Future studies could investigate the role of other modifying environmental variables in terms of *APOE* or *TOMM40* in this sample.

8.8.5. The biological interaction between TOMM40 and APOE

Greater understanding is required into the biological significance of the *APOE* ε and *TOMM40* poly-T repeat loci and their interaction. In one case, Bekris et al. (2011; N = 32) conducted functional analysis of different poly-T repeat haplotypes on reporter assay expression levels in SHSY5Y, HepG2 and U118 neuronal cell lines. Results indicated that specific *TOMM40* 523 haplotypes were associated with lower *TOMM40* – but not *APOE* – gene expression in SHSY5Y neuronal cell lines only, indicating a role for this locus in *TOMM40* promoter silencer/enhancer activity, but that the direction of its effect on expression depends on the cell type and specific haplotype content.

Chapter 4 reported nominally significant deleterious effects of the *TOMM40* 523 Short allele in specific brain MRI white matter tracts, particularly in a subgroup of participants with the *APOE* ε3/ε4 genotype. That report did not have the relevant data to discuss what may have underpinned the effects that were found (e.g. mRNA expression).

Further study of *TOMM40* poly-T repeat function and significance is therefore required in samples with relevant AD diagnostic (e.g. MMSE score), *APOE* protein/genotype, regional brain imaging and mitochondrial function data. For example, a study could examine 1) colocalization patterns between ApoE and Tomm40 proteins and/or 2) whether *APOE*/*TOMM40* genotypes show significant association/interact in affecting metrics of mitochondrial function and morphology (similar to Hedskog et al., 2012; Amy Reeve, Newcastle University Institute for Ageing and Health – personal communication).

8.9. Overview

This PhD thesis investigated the effects of variation at two specific linked gene loci that have been associated with significantly increased risk of AD – *APOE* ε and the *TOMM40* 523 poly-T repeat. Chapters 4-6 found significant, raw nominal deleterious independent effects of *APOE* ε4 and *TOMM40* 523 Short alleles on specific measures of white matter microstructural integrity but not hippocampal volumes, white matter lesions, or cerebral microbleeds. Chapter 7 showed that *APOE* ε but generally not *TOMM40* 523 was significantly associated with cognitive ability at age 73 adjusted for age 11 intelligence (i.e. cognitive ageing; Deary et al., 2004), and these associations were partially – but not completely - mediated by specific white matter tracts. Several findings became non-significant when corrected for multiple testing, but this may be overly conservative when testing inter-correlated phenotypes; on balance, replication in independent samples is warranted.

8.9.1. What contributions do the analyses in this PhD thesis make?

The findings in this PhD thesis contribute a significant amount of data to the literature on these genetic/imaging variables in generally healthy, community dwelling older adults. In certain cases, no significant association was found between *APOE* and imaging phenotypes where - previously - significant associations had been reported in independent studies. (This applies to *TOMM40* 523 to a lesser extent, on which far less previous research has been conducted). There are several possible reasons for discrepancies. Several prior reports examined relatively small samples; these are less likely to be reliable and may report spurious results. Several studies reported wide age ranges, and failed to consider important covariates such as cardiovascular disease history. There were also study-specific limitations e.g. failure to control for general brain atrophy.

These findings help to elucidate the effects of the *TOMM40* 523 poly T repeat on brain imaging and cognitive phenotypes, independent of *APOE* ε. The significant effect of *TOMM40* 523 on white matter tract integrity - but not other imaging/cognitive phenotypes - suggests that *TOMM40* 523 genotype should be considered in future studies of *APOE* ε, but also that it is generally not as influential as *APOE* ε4. The finding that *TOMM40* 523 significantly affected white matter tract integrity - but not cognitive ageing – also possibly suggests that this imaging phenotype is more sensitive to genetic effects compared with the other imaging phenotypes examined in this thesis - at least in this sample, around the age of 73 years.

Finally, the findings in this PhD help elucidate the anatomical substrates of genetic associations with cognitive ageing – namely partly but not entirely via white matter tract integrity. This research contributes to understanding of how risk factors for AD affect cognitive ageing and brain structure in older adults.

Future research should investigate, on the basis of these findings: 1) the extent to which other relevant brain imaging phenotypes mediate *APOE*-cognitive ageing associations; 2) the functional significance of *APOE/TOMM40* loci in terms of protein colocalization and mitochondrial function; 3) the role of other environmental/genetic variables in moderating *APOE*/*TOMM40* genetic effects.

8.9.2. Conclusion

On the basis of the available data in the LBC1936 (around the age of 73 years), the *APOE* ε and - to a lesser extent - *TOMM40* 523 gene loci are significant moderators of specific structural brain and cognitive ageing phenotypes in generally healthy, community-dwelling older adults. However in the case of several phenotypes such as cross-sectional hippocampal volume, previous independent reports may have overstated associations for various reasons. The genetic contributions of the *APOE* locus to cognitive ageing appear partially – but not entirely - mediated by white matter tract integrity in this sample; in this regard, future studies should investigate a broad range of structural and functional brain imaging phenotypes.

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Appendices

Appendix A – Neurocharge protocol for hypertensive status

----- Forwarded message from lorna.lopez@ed.ac.uk -----
Date: Fri, 04 Jan 2013 14:49:01 +0000
From: Lorna Lopez <lorna.lopez@ed.ac.uk>
Subject: Re: Couple of questions
To: Donald Lyall <Donald.Lyall@ed.ac.uk>

[…]

2) John Starr advised the following for me when dealing with LBC blood pressure measurements

LBC1936: unweighted mean of the second and third readings of sitting blood pressure, except for three individuals where the mean of the first and second readings were used as a third reading was not available

In LBC1936, blood pressure was measured three times in a sitting position using the B026 Omron 705IT monitor.

LBC1921: blood pressure was measured once in a sitting position.

There is also information available on which individuals are on antihypertensive medication as this is important to consider. "The blood pressure traits were adjusted for medication as suggested: For all individuals taking antihypertensive or blood pressure lowering medication, add [10]mmHg to the measured SBP value, and add [5]mmHg to the measured DBP value. For individuals not taking such medication, the imputed values are left equal to the measured values. "

I will forward this to you.

Let me know if you have any questions,

Lorna